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Dilute Acetic Acid Exposure Enhances Electrolyte Leakage by *Hydrilla verticillata* and *Potamogeton pectinatus* Tubers

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ABSTRACT

Subterranean vegetative propagules are important life cycle stages for some species of rooted aquatic plants. Sediments contain numerous compounds resulting from anaerobic degradation of organic matter, including acetic acid. Tubers of hydrilla *(Hydrilla verticillata* (L.f.) Royle) and sago pondweed (*Potamogeton pectinatus* L.) were exposed to a range of acetic acid concentrations (0, 17.4, 87, 174, 348, and 696 mmol l^{-1}) for 1, 2, 4, or 8 days. Total electrolyte leaked increased with exposure to increased acetic acid concentrations and to a lesser extent longer exposure periods for both sago pondweed and hydrilla tubers. Logistic regression of tuber survival versus total electrolyte leaked indicated that 50% of sago pondweed tubers would not survive the damage indicated by a total electrolyte leaked value of 4.5%. For hydrilla, a similar value is 12.3% Twenty amino acids were released by tubers into the external medium following acetic acid exposure. Alanine, arginine, and γ-amino butyric acid were the dominant amino acids lost from treated hydrilla tubers, accounting for 69% of the total amino acids leaked.

Key words: vegetative propagules, pondweed, aquatic plant management, amino acid.

INTRODUCTION

Submersed aquatic plants are important components of freshwater ecosystems, providing food and cover for animals and influencing physical and chemical aspects of water and sediment (Carpenter and Lodge 1986). Conversely, their abundant growth may cause water quality and utilization problems (Pieterse and Murphy 1990). Many submersed aquatic plants rely on subterranean vegetative propagules, such as tubers, turions, or winter buds for reproduction (Sculthorpe 1967). For many species, these propagules enable the plant to survive periods of unfavorable conditions (Yeo 1965, van Wijk 1988). It may be possible to develop new methods for managing aquatic weeds by attacking this critical phase of the plants life cycle (van Vierssen 1990). Spencer and Ksander (1995) exposed vegetative propagules to acetic acid concentrations from 0 to 696 mmol l^{-1} for 1 to 8 days. They reported that hydrilla propagules were more sensitive to acetic acid exposure than those of either variable pondweed *(Potamogeton gramineus* L.) or sago pondweed. Monoecious hydrilla propagules did not sprout at acetic acid concentrations ≥ 17 mmol l^{-1} even at the shortest exposure time, one day.

To learn more about the effects of acetic acid on aquatic plant propagules we measured electrolyte leakage by sago pondweed and monoecious hydrilla tubers following exposure to acetic acid in this study. Electrolyte leakage is one of a number of physiological parameters that have been used as rapid indicators of exposure of plants to specific stresses. It has been shown that stresses such as chilling, freezing, heat, or desiccation lead to an increase in passive ion efflux due to a decrease in membrane integrity (Hendry and Grime 1993).

More recently, Sprecher and Netherland (1995) have called for relating easily measured indicators of plant stress to whole plant survival. Their aim was to develop relationships between rapidly measured physiological responses and plant survival so that exposure of target and non-target plants could be monitored following aquatic herbicide applications. For this reason, a second objective was to develop a mathematical relationship between electrolyte leakage by tubers and their likelihood of survival following exposure to acetic acid. A third objective was to characterize in part the electrolytes lost by acetic acid treated tubers. This information may explain the differential tolerance to acetic acid exposure by aquatic plant propagules previously reported or it may provide a basis for developing methods for detecting damaged tubers in the field.

MATERIALS AND METHODS

Experiment 1: Tubers from sago pondweed and monoecious plants of hydrilla were collected from cultures maintained at the USDA Aquatic Weed Control Research Laboratory, Davis, California, for experimental use. Sago pondweed tubers were harvested the day the experiment started. Hydrilla tubers were harvested and the experiment started the second day after harvest. During that period tubers were kept at 4 C in a sealed plastic bag. To avoid variation due to tuber size differences, tubers were selected so that the fresh weights were between 100 and 150 mg for both species. Tubers were exposed to a range of acetic acid concentrations (0, 17.4, 87, 174, 348, and 696 mmol l^{-1}) by placing them in a flask containing 200 ml of the appropriate

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acetic acid solution for 1, 2, 4, or 8 days. Following exposure, tubers were removed from the acetic acid solution, rinsed with deionized water, and individually placed in a 50-ml plastic centrifuge tube containing 25 ml of deionized water. The initial conductivity of the water was measured using a digital conductivity meter (Model 09326-2, Fisher Scientific, Pittsburgh, PA). One hour later the conductivity was again measured. Following this measurement, the test tubes containing the tubers were placed in a boiling water bath for 10 minutes. Test tubes were then covered and allowed to stand at room temperature for an additional 24 hours. Then a final conductivity reading was taken. Total electrolyte leaked (%) was calculated according to the following equation: total electrolyte leaked = (conductivity at 1 hour - initial conductivity)/conductivity 24 h after boiling. This follows Hendry and Grime's (1993) procedure. Since previous results (Spencer and Ksander 1995) indicated a nonlinear relationship for survival versus acetic acid concentration and exposure time, we combined the data for all times and acetic acid exposures and used nonlinear regression techniques (SAS Institute Inc. 1989) to fit them to a Michaelis-Menten model with total electrolyte leaked as the response variable and acetic acid concentration as the independent variable. Employing this approach, we estimated maximum total electrolyte leaked and the acetic acid concentration which produced one half of the maximum total electrolyte leaked. Confidence intervals for these parameters were estimated and used to compare species' responses.

To relate tuber survival to total electrolyte leaked we combined the data from this experiment with survival data published by Spencer and Ksander (1995) for tubers treated in precisely the same manner described above. We analyzed the data by logistic regression (Cox and Snell 1989, Collette 1991) with survival as the dependent variable and total electrolyte leaked as the independent variable (SAS Institute Inc. 1989).

Experiment 2: The following experiment was performed to characterize the electrolytes leaked by acetic acid treated tuber. Ten tubers of monoecious hydrilla (135 to 165 mg fresh weight) and 10 sago pondweed tubers (45 to 65 mg) were harvested from cultures. Ten ml of distilled water were added to 2 scintillation vials, serving as controls. Ten ml of a 174 mmol l^{-1} solution of acetic acid was added to 2 other vials. A 50-µl sample was taken out of each vial, representing conditions at time zero. Tubers were then added, 5 in the control and 5 in the treated for each species. The contents of each vial were stirred and than another 50-µl sample was taken at 5, 10, 15, 30, and 60 minutes. Sampling continued hourly, during work hours (8-5) for the next 48 hours. Each microfuge tube was labeled, capped, and stored at 4 C for future processing. After 48 hours, tubers were removed from the solutions and dried at 80 C for 48 hours. The presence of total amino acids was determined using the ninhydrin assay (Moore and Stein 1948) with leucine as a standard. This experiment was repeated.

The remaining contents of the vials were frozen (-70 C) for later amino acid analysis. Amino acid analysis was performed by the Protein Structure Laboratory, University of California, Davis, CA. A brief description of the procedures employed follows. Amino acid composition was analyzed on a Beckman 6300 amino acid analyzer with a lithium citrate buffer system. Amino acids were separated by ion-exchange chromatography. A post-column ninhydrin reaction yielded a colored complex, the intensity of which was monitored at two wavelengths. Beckman System Gold software identified and quantified the amino acids present.

RESULTS

Total electrolyte leaked increased with exposure to increased acetic acid concentrations or longer exposure periods for both sago pondweed and hydrilla tubers (Figure 1; Table 1). For sago pondweed, the maximum total electrolyte leaked was 19.4% with 95% confidence limits of 14.8 to 24%. The acetic acid concentration which produced one half the maximum electrolyte leaked was 157.3 mmol l^{-1}. The 95% confidence interval was 50.7 to 263.8 mmol l^{-1} acetic acid. Hydrilla maximum total electrolyte leaked was estimated as 47.9% with 95% confidence limits of 41.3 to 54.5%. The acetic acid concentration producing one-half the maximum total electrolyte leaked was 27.1 mmol l^{-1}. The 95% confidence intervals for this parameter were 6.7 to 47.4 mmol l^{-1} acetic acid. These results indicate that hydrilla tubers were more susceptible to damage by exposure to lower concentrations of acetic acid.

Figure 2 shows the relationships between tuber survival and electrolyte leakage. Logistic regression equations describing this relationship were significant (Table 2) indicating that the probability of survival for tuber could be predicted by characterizing the total electrolyte leaked for the tuber. Logistic regression results indicate that 50% of sago pondweed tubers would not survive the damage indicated by a total electrolyte leaked value of 4.5%. For hydrilla, a similar value is 12.3%.

Figure 3 shows that amino acids leaked from hydrilla tubers exposed to 174 mmol l^{-1} acetic acid for 48 hours. Amino acid leakage from tubers exposed to deionized water was less. Leakage increased most during the first 10 hours following the acetic acid exposure. Amino acids also leaked from sago pondweed tubers exposed to acetic acid relative to controls (Figure 3). The rate of amino acid leakage was less than that observed for hydrilla tubers.

Twenty amino acids were detected in the external medium of acetic acid treated tubers (Table 3). The quantity of amino acids leaked from hydrilla tubers exposed to 174 mmol l^{-1} acetic acid for 48 hours was about 7 times that of tubers exposed to deionized water. Alanine, arginine, and γ-amino butyric acid were the dominant amino acids lost from treated hydrilla tubers, accounting for 69% of the total amino acids leaked. For sago pondweed tubers, the amount of amino acids released by acetic acid exposed tubers was greater than that of those incubated in deionized water. No amino acids were detectable in the external medium of the deionized water incubated tubers. The three most abundant amino acids leaked from treated sago pondweed tubers were arginine, g-amino butyric acid, and alanine. The total amount of amino acids leaked from treated sago pondweed tubers was about 3 times that leaked from the hydrilla tubers exposed deionized water.

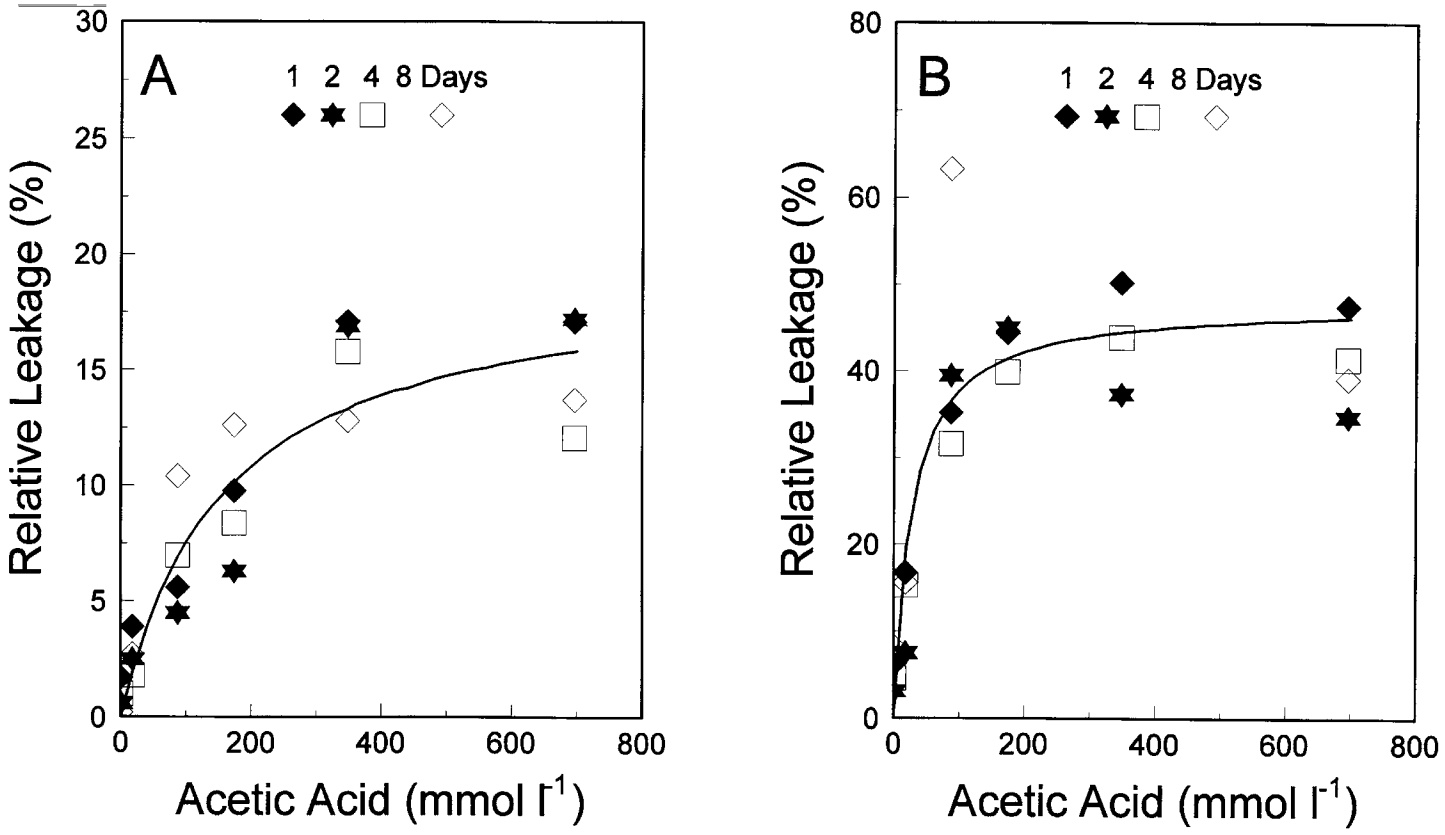

Figure 1. Response of sago pondweed (A) and hydrilla (monoecious biotype) (B) tubers to exposure to acetic acid for 4 time intervals. The solid lines were fitted by nonlinear regression. Resulting equations are listed in Table 1.

TABLE 1. PARAMETERS FOR AN EQUATION DESCRIBING TOTAL ELECTROLYTE LEAKED AS A FUNCTION OF ACETIC ACID CONCENTRATION FOR HYDRILLA AND SAGO PONDWEED TUBERS. THE EQUATION HAS THE FORM $TEL = TEL_{MAX} * AA/(AA_{1/2} + TEL_{MAX})$ WHERE TEL IS THE TOTAL ELECTROLYTE LEAKED (%); TEL_{MAX} IS THE ESTIMATED MAXIMUM TOTAL ELECTROLYTE LEAKED; AA IS THE ACETIC ACID CONCENTRATION (MMOL L^{-1}), $AA_{1/2}$ IS THE ACETIC ACID CONCENTRATION PRODUCING ONE HALF OF THE MAXIMUM TOTAL ELECTROLYTE LEAKED.

Plant	TEL_{max}	Standard Error	$AA_{1/2}$	Standard Error
Sago pondweed	19.39	2.22	157.20	51.40
Hydrilla	47.93	3.16	27.08	9.82

DISCUSSION

Electrolytes leaked from tubers exposed to acetic acid, and the quantity leaked increased with increased concentration of the acetic acid in a nonlinear fashion. The percentage of total electrolytes leaked was significantly greater for hydrilla than for sago pondweed. As measured by the acetic acid concentration producing one-half of maximum electrolyte leakage Hydrilla propagules were about six times more sensitive to acetic acid exposure than sago pondweed propagules. This result agrees with earlier findings on tuber survival reported by Spencer and Ksander (1995). Increased electrolyte leakage by acetic acid exposed tubers indicates that part of the mechanism leading to death (defined here as non-sprouting and subsequent rapid decomposition) of acetic acid treated tubers involves the disruption of cell membrane integrity.

The probability of survival for acetic acid treated tubers was predictable from measurements of total electrolyte leaked. Based on values for Somers' D and the gamma statistic, both of which measure the correlation between predicted and observed data, this relationship was stronger for hydrilla than for sago pondweed. The relationships developed here should be useful in rapidly assessing the impact of acetic acid applications to natural tuber populations.

The present results do not directly address the importance of naturally formed acetic acid on the survival of tubers in sediments, but may be of applied significance. Acetic acid levels in a given sediment depend on the availability of organic matter and vary with season and depth. Reeburg (1983) reported acetic acid concentrations from 0.1 to 360 µmol l^{-1} for marine and freshwater sediments. Miller et al. (1979) reported acetic acid concentration of 2.5 to 8.6 mmol l^{-1} in pore water from Loch Eil sediments amended with cellulose. When a soil is flooded, the concentration of volatile organic acids (predominantly acetic acid) may increase to 40 mmol l^{-1} within 1 to 2 weeks, and then decline to less than 1 mmol l^{-1} within a few weeks (Ponnamperuma 1972). Because it is an important intermediate in sediment microbial metabolism, acetic acid may have a rapid turnover time (hours to a few days; see Reeburg (1983)). Thus manipulating either the metabolism of acetic acid or its concentration in the sediment may be useful in managing tuber survival in given habitat. In addition, the present results indicate that for managing hydrilla tubers, the manipulation of either aspect may not have to

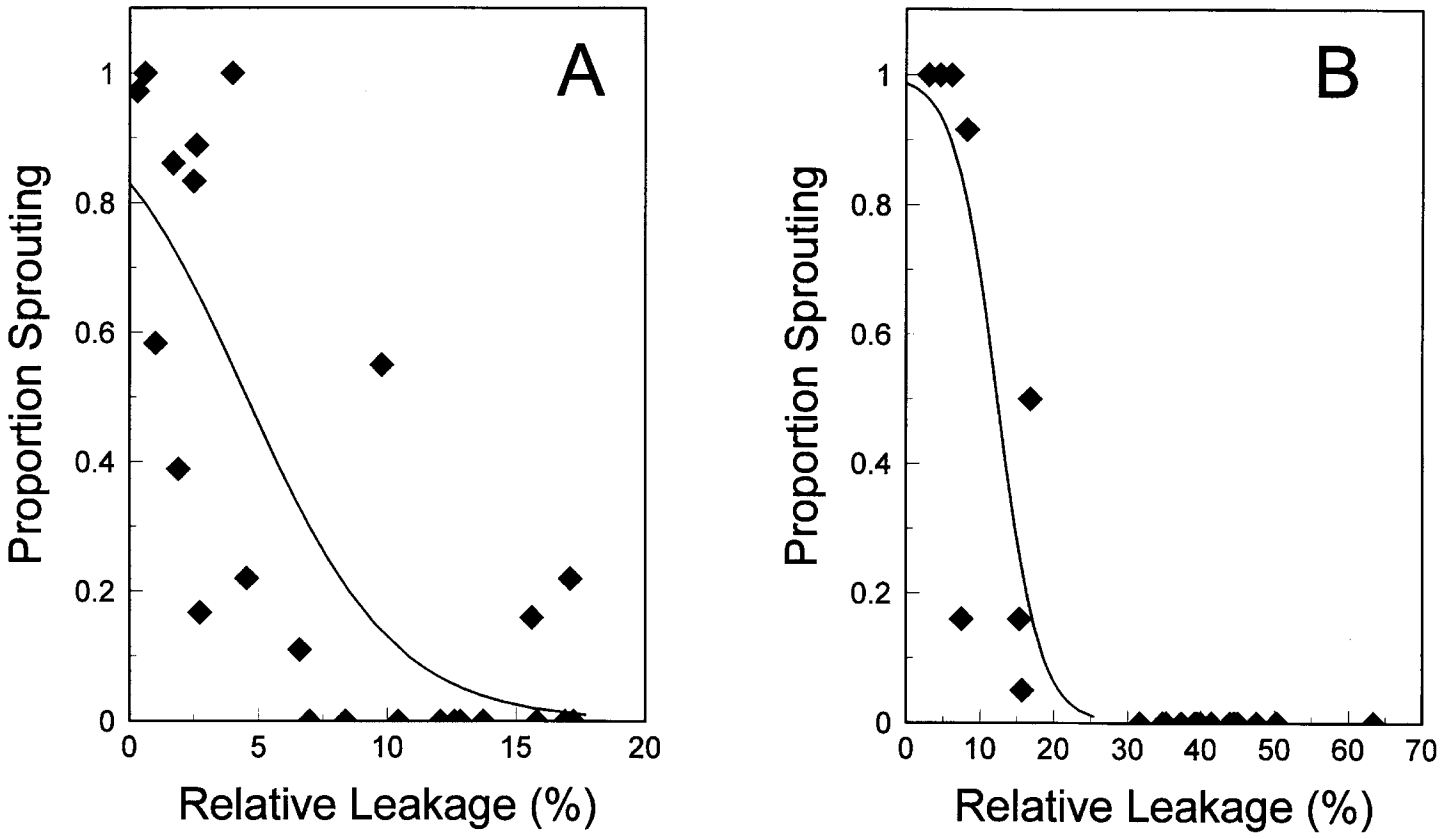

Figure 2. Relationships between survival and total electrolyte leakage for sago pondweed (A) and monoecious hydrilla (B) tubers. Lines fitted by logistic regression. See Table 2 for equations.

TABLE 2. RESULTS OF LOGISTIC REGRESSION RELATING THE PROPORTION NOT SPROUTING TO TOTAL ELECTROLYTE LEAKED (TEL) FOR HYDRILLA AND SAGO PONDWEED TUBERS. SOMERS' D AND GAMMA SHOW THE CORRELATION BETWEEN PREDICTED VALUES AND ACTUAL VALUES. S.E. IS THE STANDARD ERROR OF THE ESTIMATED PARAMETER. MODEL SIGNIFICANCE SHOWS THE RESULTS OF A WALD CHI-SQUARE TEST WITH 1 DF. IN THIS CONTEXT, THE LOGISTIC EQUATION HAS THE FORM: $P = E^{LOGIT}/(1 + E^{LOGIT})$, WHERE P IS THE PROBABILITY THAT A TUBER WILL NOT SPROUT; LOGIT = INTERCEPT + (COEFFICIENT* TEL); THE VALUES FOR INTERCEPT AND COEFFICIENT ARE GIVEN IN THE ESTIMATE COLUMN BELOW.

Variable	Estimate	Standard Error
Sago pondweed		
Intercept	1.59	0.18
Total Electrolyte Leaked (%)	-34.99	3.14
Model Significance	0.0001	
Somers' D	0.79	
Gamma	0.80	
Hydrilla		
Intercept	4.35	0.49
Total Electrolyte Leaked (%)	-0.35	0.04
Model Significance	0.0001	
Somers' D	0.96	
Gamma	0.96	

be of a large magnitude relative to naturally occurring fluctuations in either turnover time or concentration.

The types and quantities of amino acids leaked by acetic acid exposed tubers were consistent with the scant published

TABLE 3. IDENTIFICATION OF AMINO ACIDS (NMOLES/ MG) LEAKED FROM TUBERS EXPOSED TO 174 MMOL L^{-1} ACETIC ACID OR DEIONIZED WATER FOR 48 HOURS.

	Hydrilla tubers		Sago pondweed tubers	
Amino Acid	Acetic Acid	Deionized Water	Acetic Acid	Deionized Water
Aspartic Acid	2.19	0.06	2.73	0
Threonine	0.52	0.05	0.64	0
Serine	0.68	0.05	2.78	0
Asparagine	3.48	0.20	1.95	0
Glutamic Acid	3.15	0	0.75	0
Glutamine	3.25	0	0.95	0
Proline	0.78	0.10	0	0
Glycine	1.42	0.19	1.04	0
Alanine	30.79	2.73	3.54	0
Valine	0.37	0.19	0.94	0
Cysteine	0.14	0.01	0.51	0
Methionine	0.06	0.05	0.10	0
Isoleucine	0.20	0.09	0.58	0
Tyrosine	0.09	0.02	0.34	0
Leucine	0.36	0.13	0.54	0
Phenylalanine	0.34	0.05	0.45	0
γ-Amino Butyric Acid	16.93	1.25	10.74	0
Lysine	1.45	0.08	1.52	0
Histidine	2.46	0.13	0.98	0
Arginine	29.25	0.00	11.86	0
Total	97.91	5.38	42.94	0

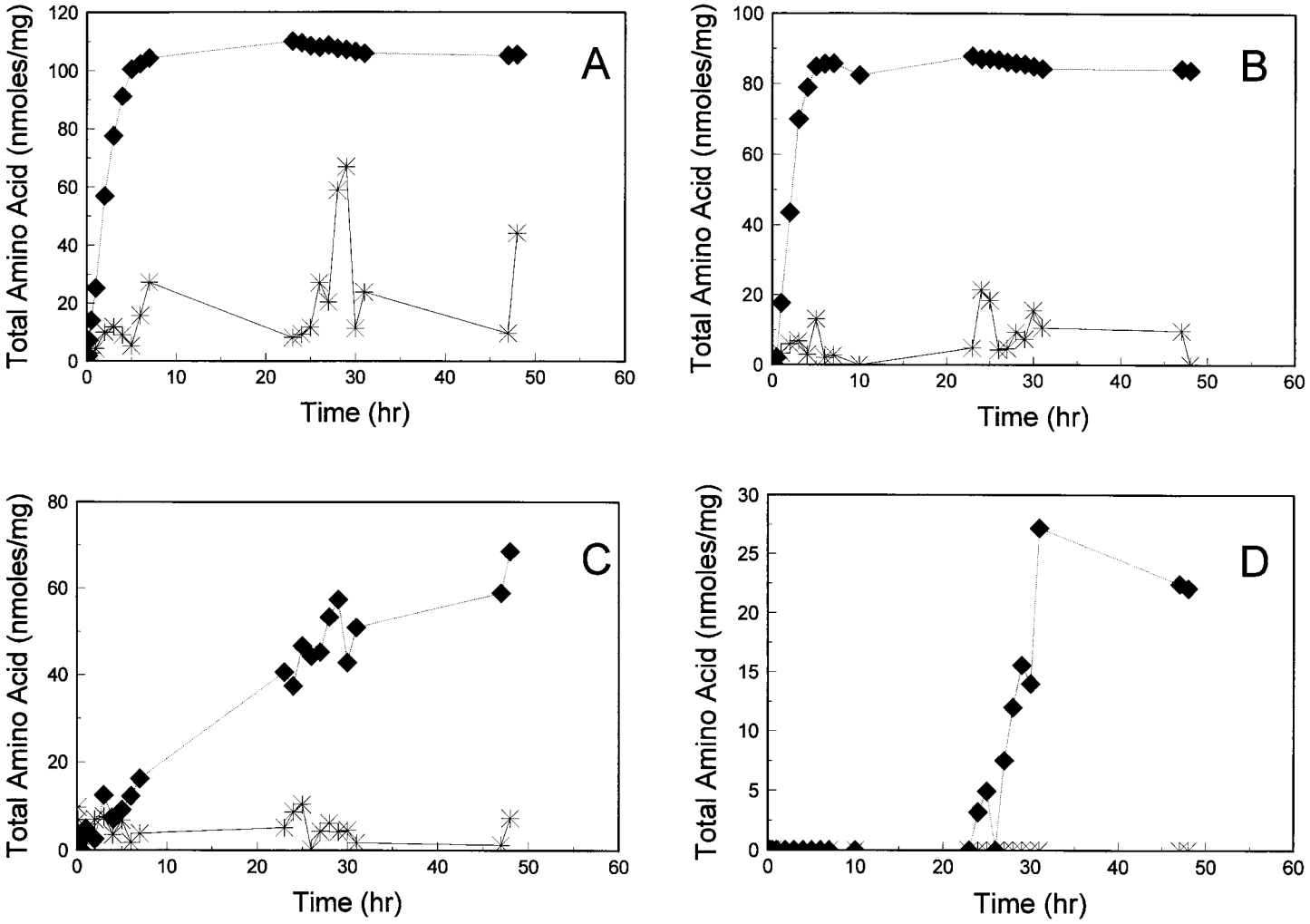

Figure 3. Time course of total amino acids leaked by hydrilla tubers (A and B) and sago pondweed tubers (C and D) exposed to 174 mmol l^{-1} acetic acid for 48 hours in two experiments (solid diamonds) or controls (deionized water, asterisks).

data on tuber amino acid composition. Ryan (1994) reported that hydrilla tubers contained from 20 to 70 nmoles of amino acids mg^{-1}. The levels of amino acid leaked by hydrilla tubers in the present study was from 1 to 1.5 times the level reported by Ryan (1994). This represents relatively close agreement since it is not clear that the amino acid pools are static. On the other hand, it is possible that acetic acid exposure may lead to protein degradation. The types of amino acids leaked from tubers in this study were similar to those reported by Ryan (1994) for hydrilla tubers. Ryan (1994) reported that the principal free amino acids in ethanolic extracts of monoecious hydrilla tubers were asparagine, alanine, and arginine, and that γ-amino butyric acid was present in quantities similar to those of serine.

Underground vegetative structures are important to the long term survival of some species of aquatic weeds. Collectively these structures comprise the propagule bank which in some ways is analogous to a seed bank. Results of this study offer an easily measured rapid way to assess tuber health and thus may be directly applicable to developing novel methods for managing aquatic weeds by manipulating propagule behavior in sediment. Kremer (1993) has proposed that weed seed banks may be depleted by integration of methods which incorporate microorganisms or their metabolites into the soil to "attack" seeds. The present results demonstrate that propagules of the invasive aquatic plant hydrilla are more sensitive to the microbial metabolite, acetic acid, than those of a native species, sago pondweed. As suggested by Kremer (1993), exploitation of ecological aspects of weed propagule - microorganism - sediment environment relationships may lead to progress toward effective weed management systems.

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