J. Aquat. Plant Manage. 37: 80-82

NOTES

Effect of Cattail (*Typha domingensis*) Extracts, Leachates, and Selected Phenolic Compounds on Rates of Oxygen Production by Salvinia (*Salvinia minima*)

MARIA T. GALLARDO[1], JENNIFER R. ASCHER[1], M. JORDAN COLLIER[1], BARBARA B. MARTIN[1] AND DEAN F. MARTIN[1,2]

INTRODUCTION

Salvinia (*Salvinia minima* Willd.) is a water fern found in Florida waters, usually associated with *Lemna* and other small free-floating species. Due to its buoyancy and mat-forming abilities, it is spread by moving waters. In 1994, salvinia was reported to be present in 247 water bodies in the state (out of 451 surveyed public waters, Schardt 1997). It is a small, rapidly growing species that can become a nuisance due to its explosive growth rates and its ability to shade underwater life (Oliver 1993). Any efforts towards management of salvinia populations must consider that, in reasonable amounts, its presence is desirable since it plays an important role in the overall ecosystem balance. New management alternatives need to be explored besides the conventional herbicide treatments; for example, it has been shown that the growth of *S. molesta* can be inhibited by extracts of the tropical weed parthenium *(Parthenium hysterophorus*) and its purified toxin parthenin (Pandey 1994, 1996).

We believe that cattail, *Typha* spp. may be a candidate for control of *S. minima* infestations. Cattail is an aggressive aquatic plant, and has the ability to expand over areas that were previously occupied by other species (Gallardo et al. 1998a and references cited there). In South Florida, *T. domingensis* is a natural component of the Everglades ecosystem, but in many cases it has become the dominant marsh species, outcompeting other native plants. In Florida public waters, this cattail species is the most dominant emergent species of aquatic plants (Schardt 1997). Several factors enable it to accomplish opportunistic expansion, including size, growth habits, adaptability to changes in the surroundings, and the release of compounds that can prevent the growth and development of other species.

We have been concerned in the past with the inhibitory effects of the *T. domingensis* extracts, and the phenolic compounds mentioned before, towards the growth and propagation of *S. minima* (Gallardo et al. 1998b). This investigation deals with the impact of cattail materials on the rates of oxygen production of salvinia, as determined through a series of Warburg experiments (Martin et al. 1987, Prindle and Martin 1996).

[1]Institute for Environmental Studies, Department of Chemistry, University of South Florida, Tampa, FL 33620. Received for publication November 12, 1998 and in revised form July 30, 1999.

MATERIALS AND METHODS

Salvinia Culture. Salvinia (*S. minima* Willd.) was collected at Lettuce Lake (Lettuce Lake Park, Hillsborough County, FL) where it grows mixed with *Lemna* spp. and covers most of the lake shores and adjacent swamp areas. Plants were identified by Dr. Frederick Essig, at the University of South Florida (USF) Herbarium. After collection, the material was cleaned by rinsing three times under running tap water. After this, the plants were dipped in a 5% solution of commercial bleach for a minute and thoroughly rinsed with deionized water. The plants were then divided in clumps with approximately 10 fronds and kept in sterile foam-stoppered 125 ml Erlenmeyer flasks containing 50 ml of modified Hoagland's nutrient solution (Steward and Elliston 1973). The stock cultures were maintained for up to a week prior to use and were kept at 28 C under cool white light (150 $\mu E/m^2/s$), 12 h photoperiod.

Cattail Extracts. Fully mature cattail samples were taken from a storm water ditch near the University of South Florida campus in Tampa, FL. The samples were identified as *T. domingensis* by Dr. Richard P. Wunderlin, at the USF Herbarium. After collection, the samples were rinsed with deionized water and were divided into three sections: the root system (including rhizomes), the stem (taken from just above the root to the part where the leaves start separating), and the leaves. A portion (usually about 150 g) of each section was weighed and extracted with deionized water (5 ml per 1.0 g of material) at room temperature using a Hamilton Beach blender. The resulting crude extracts were then filtered using Whatman no. 1 filter paper, and the remaining liquid was then autoclaved for 1 h and placed in a refrigerator at 0 to 4 C and disposed of after two weeks (Prindle and Martin 1996).

Cattail Leachates. Fully mature cattail collected in the manner described above was rinsed under running tap water and gently brushed with a soft brush to remove soil and debris, then rinsed with distilled water. After cleaning, the cattail was clamped to a support and placed in a plastic reservoir with enough distilled water to cover its root system. The water was maintained at constant temperature by placing the reservoir in a controlled temperature bath (New Brunswick Scientific, Model G79, New Brunswick, NJ) at 25 C and the system was maintained under constant illumination by fluorescent lights placed surrounding the stem (100 $\mu E/m^2/s$ as measured by a model Li 185A Li-Cor radiometer/photometer). After a period of 24 h, the water was collected and autoclaved. The leachates prepared in this manner were kept in the refrigerator until required.

Phenolic Compounds. Phytotoxic substances known to be present in the cattail extracts were also studied. Solutions with concentrations of 10, 20, 50, and 100 mg/L of both 2-chlorophenol (Aldrich Chemical Company, Inc., Milwaukee, WI) and salicylaldehyde (Mallinckrodt, Inc., St. Louis, MO) in distilled water were prepared.

Determination of Rates of Oxygen Production. Modified Hoagland's (Steward and Elliston 1973) nutrient solution (2 mL) was pipetted into the outer annulus of Warburg flasks. A known amount of salvinia (typically 2 g fresh weight) was placed in the solution. Central wells of the Warburg flasks were filled with filter paper strips saturated with 10% aqueous KOH to absorb CO_2; the side arm contained 1ml of the extract, leachate or solution being tested. The atmosphere was N_2. Illumination was provided by fluorescent lamps arranged vertically around the water bath. Observed intensity was 100 $\mu E/m^2/s$. Water temperature was maintained at 25 C by means of a Sargent-Welsh thermoregulator.

Manometer readings were recorded every 15 min for about 1 h. Manometer readings, recorded as a function of time (min), were fitted to a straight line by a linear regression procedure. The slopes of the lines were taken as a measure, in arbitrary units, of the rate of photosynthesis under the conditions used. Once good initial rates were obtained, the contents of the flask side-arm were added to the medium, and, after a 30-min incubation period, the photosynthetic rates were measured as before. Analyses were repeated twice for each treatment.

The impact of cattail-derived materials on the rates of oxygen production by *S. minima* are summarized in Table 1. For each run, the data were subjected to regression analysis, the quality of fit being indicated by statistically significant linear correlation coefficients ($r^2 = 0.95$ or better, $P = 0.05$) for the control run, and then the same procedure was applied to the measurements made after the addition of the substance being tested. The slopes before and after treatment were compared using Student's t-test, and the differences were found to be significant at the 95% confidence level. P-values are given in Table 1. Percent decrease in the rate of oxygen production was calculated on the basis of the control for each trial.

RESULTS AND DISCUSSION

The data indicate that the production of oxygen by salvinia was inhibited by the presence of cattail extracts and leachates. For the cattail extracts, the inhibitory effect was greater for the root fraction, and the inhibitory activity decreased in extracts from the stem and the leaves, but the effect was still noticeable even in the less active fractions.

The results obtained for the cattail leachate indicate that its potency as an inhibitory agent is higher than any of the extracts that we tested. This observation is particularly interesting, because it suggests that the substances naturally released by the cattail are highly active as phytotoxic agents. The release process, which can be associated with the production of secondary metabolites, renders a more phytotoxic material than obtained from water extractions performed under laboratory conditions (Tang and Young 1982). Collection of the extracellular materials responsible for inhibition was also important because it approximates more closely the field interaction between salvinia and cattail.

When pure phenolic compounds (2-chlorophenol and salicylaldehyde) were tested for inhibition of oxygen production, a pattern of inhibition was found (see Table 1). Even the more diluted fractions, containing 1.7 to 3.3 ppm of the chemicals, were inhibitory towards salvinia. The more concentrated solutions had more effective inhibitory properties. These results suggest that the phenolic fraction is indeed responsible for the phytotoxic behavior of the cattail extracts.

The effects observed for the cattail root fraction are comparable to the effects of the 2-chlorophenol fraction (6.7 mg/L) or the salicylaldehyde fraction (3.3 mg/L); these concentrations correspond to higher amounts of the chemicals than what we have found in the cattail extracts. However, these two phenols are likely not the only components of the

TABLE 1. RATE OF OXYGEN PRODUCTION BY *SALVINIA MINIMA* IN THE PRESENCE OF AQUEOUS CATTAIL EXTRACTS AND SELECTED PHENOLIC COMPOUNDS, MEAN ± STANDARD ERROR FOR TWO DIFFERENT EXPERIMENTS.

Treatment	Initial slope	Post-treatment slope	p-value	% decrease in rate of oxygen production after addition
Cattail extract				
Leaves	0.58 ± 0.02	0.30 ± 0.01	0.04	49 ± 4
Stem	0.64 ± 0.05	0.15 ± 0.01	0.04	58 ± 1
Root	0.39 ± 0.01	0.08 ± 0.02	0.04	78.4 ± 0.8
Leachate	0.64 ± 0.05	0.04 ± 0.03	0.04	93 ± 5
2-chlorophenol Effective Concentration (mg/L)				
3.3	0.34 ± 0.07	0.18 ± 0.04	0.05	49 ± 2
6.7	0.46 ± 0.06	0.06 ± 0.03	0.02	87 ± 6
16.7	0.65 ± 0.08	0.02 ± 0.01	0.04	98 ± 1
33.3	0.36 ± 0.06	0.006 ± 0.004	0.05	99 ± 1
Salicylaldehyde Effective Concentration (mg/L)				
1.7	0.62 ± 0.09	0.20 ± 0.01	0.03	68 ± 4
3.3	0.47 ± 0.07	0.08 ± 0.01	0.04	80.9 ± 0.5
6.7	0.64 ± 0.04	0.081 ± 0.008	0.02	87 ± 2
16.7	0.6 ± 0.1	0.02 ± 0.01	0.04	97 ± 2
33.3	0.49 ± 0.06	0.009 ± 0.005	0.03	98 ± 1

cattail extracts. While our work has been done using aqueous extracts of *T. domingensis*, organic extracts of *T. latifolia* have been studied by other groups, and some other phytotoxins have been isolated. These include certain steroids and fatty acids (Alliotta et al. 1990), several free and acylglucosylated stigmasterols (Della Greca et al. 1990a), and (20S)-4-methylenecholest-7-en-3-ol (Della Greca et al. 1990b). However, the availability of these compounds obtained by organic-solvent extraction in the natural cattail environment is open to question, since the extraction conditions are far removed from the actual release of secondary metabolites in the field. The likelihood that these materials would be widely extracted with pond water, should they be present in *T. domingensis*, seems doubtful. Nevertheless, the existence of other phytotoxic materials is still under study.

It has been suggested (Pandey 1994a) that loss of membrane integrity and disruption of enzymatic pathways are possible ways of action for phenolic-type phytotoxins. Our results, both for these oxygen production studies and for previous work on growth and propagation of salvinia in the presence of cattail (Gallardo et al. 1998b), are in good agreement with the proposed action mode.

ACKNOWLEDGMENTS

We are grateful to Dr. Frederick Essig and Dr. Richard P. Wunderlin, both at the USF Institute for Systematic Botany and USF Herbarium for the identification of plant specimens. We thank Michael Arias, USF Scientific Glassblower for making the Warburg flasks. We appreciate the helpful comments of Dr. John D. Madsen and our reviewers.

LITERATURE CITED

Alliotta, G., M. Della Greca, P. Monaco, G. Pinto, A. Pollio and L. Previtera. 1990. *In vitro* algal growth inhibition by phytotoxins of *Typha latifolia*. J. Chem. Ecol. 16: 2637-2646.

Della Greca, M., P. Monaco, and L. Previtera. 1990a. Stigmasterols from *Typha latifolia*. J. Nat. Prod. 53: 1430-1435.

Della Greca, M., L. Mangoni and A. Molinaro. 1990b. (20S)-4-methylenecholest-7-en-3-ol, an allelopathic sterol from *Typha latifolia*. Phytochemistry 29: 1797-1798.

Gallardo, M. T., B. B. Martin and D. F. Martin. 1998a. An annotated bibliography of allelopathic properties of cattails, *Typha spp.* Florida Scient. 61(1): 52-58.

Gallardo, M. T., B. B. Martin and D. F. Martin. 1998b. Inhibition of Salvinia (*Salvinia minima*) by cattail (*Typha domingensis*) extracts and by 2-chlorophenol and salicylaldehyde. J. Chem. Ecol. 24: 1483-1490.

Martin, B. B., D. F. Martin and M. J. Perez-Cruet. 1987. Effect of selected dyes on the growth of the filamentous blue-green alga, *Lyngbya majuscula*. J. Aquat. Plant Manage. 25: 40-3.

Oliver, J. D. 1993. A review of the biology of the giant *Salvinia* (*Salvinia molesta* Mitchell). J. Aquat. Plant Manage. 31: 227-231.

Pandey, D. K. 1994a. Inhibition of Salvinia (*Salvinia molesta* Mitchell) by Parthenium (*Parthenium hysterophorus* L.). I. Effect of leaf residue and allelochemicals. *J. Chem. Ecol.* 20: 3111-3122.

Pandey, D. K. 1996. Phytotoxicity of sesquiterpene lactone parthenin on aquatic weeds. J. Chem. Ecol. 22: 151-160.

Prindle, V. and D. F. Martin. 1996. Allelopathic properties of cattails, *Typha domingensis*, in Hillsborough County, Florida. Florida Scient. 59: 155-162.

Schardt, J. D. 1997. 1994 Florida Aquatic Plant Survey. Dominant Plants in Public Waters. Tech Report #972-CGA. Bureau of Aquatic Plant Management. Florida Department of Environmental Protection. Tallahassee, FL. 85 pp.

Steward, K. K. and R. A. Elliston. 1973. Growth of hydrilla in solution culture at various nutrient levels. Florida Scient. 36: 228-233.

Tang, C. S. and C. C. Young. 1982. Collection and identification of allelopathic compounds from the undisturbed root system of Bigalta Limpograss (*Hemarthria altissima*). Plant Physiol. 69: 155-160.