

Coexistence of Monoecious and Dioecious Hydrilla in Lake Gaston, North Carolina and Virginia

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ABSTRACT

Biotypes of hydrilla (*Hydrilla verticillata* (L.f.) Royle) at two sites in North Carolina were characterized by growth habit and flower type under controlled conditions, patterns of tuber proteins after electrophoresis, and reaction of DNAs with a single primer in the random amplified polymorphic DNA (RAPD) reaction. Plants from Burnt Mill Creek in Wilmington, in the southeastern part of the state, were dioecious by all criteria. Plants of both the monoecious and dioecious biotype were found in Lake Gaston, at the Virginia-North Carolina border. This is the first report of the presence of plants of both biotypes in a single body of water.

Key words: biotype, electrophoresis, growth habit, RAPD, tuber proteins.

INTRODUCTION

Two biotypes of hydrilla are presently found in the United States, of the 23 reported world-wide (Verkleij et al. 1983, Verkleij and Pieterse 1991). A female dioecious plant was introduced in Florida in the mid- to late-1950s (Schmitz et al. 1990), and since then, has spread throughout the southeastern states, including Georgia, Alabama, and South Carolina, and is now reported as far west as Texas and California. A plant of the monoecious biotype was described in lakes in North Carolina (Langeland and Schiller 1983) and the Potomac River near Washington D. C. (Steward et al. 1984). A monoecious plant, presumably the same one as described above, has been reported in Maryland and Delaware (Verkleij and Pieterse 1986). A monoecious plant has been noted as well in Lake Gaston³, an impoundment of the Roanoke River at the North Carolina-Virginia border.

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³Langeland, K. A., and D. J. Pascretta 1986. Distribution, composition, and chemical control of the submerged aquatic vegetation in Lake Gaston, Virginia-North Carolina. Annual Report to the Virginia Electric Power Company. 43 pp.

⁴Mention of a product or company name does not constitute an endorsement or guarantee by the USDA or the North Carolina Agricultural Research Service and does not imply a recommendation to the exclusion of other similar products.

Until now there have been no reports of the monoecious and dioecious biotypes coexisting within the same body of water in the U.S. In the fall of 1992, hydrilla was noted in the Virginia section of Lake Gaston that was different in appearance from the established monoecious plant; this plant had longer leaves, shorter internode distances and apparently heavier pigmentation than the monoecious plant growing in close proximity. The new plant was more robust in appearance than the established biotype.

Hydrilla has been noted since 1989 in Burnt Mill Creek, in Wilmington, North Carolina (Chuck Wilson, U. S. Army Corps of Engineers, Wilmington, NC, personal communication). This was identified as the dioecious biotype by one of the authors (S. H. K.), by Dr. David DeMont, North Carolina Aquatic Weed Program, and by Dr. Lars Anderson, USDA ARS Aquatic Weed Laboratory, Davis, CA, based on morphological criteria (personal communication).

This work was undertaken to determine the biotype of the new infestation of hydrilla in Lake Gaston and to confirm that in Burnt Mill Creek.

MATERIALS AND METHODS

Subterranean turions (Pieterse 1983), hereafter called tubers, were collected during the fall and winter from Lake Gaston and from Burnt Mill Creek by sieving the hydrosoil in the areas of the suspected dioecious plants. Collection sites are indicated in Figure 1. Tubers were also collected from areas of Lake Gaston that support the monoecious biotype: these served as controls and as voucher specimens. For comparison, tubers of dioecious plants were provided by Dr. Ken Langeland from cultures at the University of Florida, Gainesville, FL. In order to compare growth habits and induce flowering, plants were grown in the phytotron at North Carolina State University, Raleigh, NC. Tubers collected from Burnt Mill Creek, from the monoecious and suspected dioecious plants from Lake Gaston, and from dioecious plants from the University of Florida were used. Three tubers from the same site were planted in a 114-L aquarium with 12.5 cm of loam hydrosoil from Lake Gaston, and a total of four aquaria were used. Each aquarium was equipped with a filter that filtered 454 L /h. Aquaria were initially filled with filtered pond water, and volumes were maintained with distilled water. A 9-h photoperiod was used with a day temperature of 26 C and a night temperature of 22 C. A photosynthetic photon flux density of 700 $\mu\text{mol m}^{-2}\text{s}^{-1}$ was produced by a combination of fluorescent and incandescent lights. The study began on April 20, 1993, and was terminated on August 20,

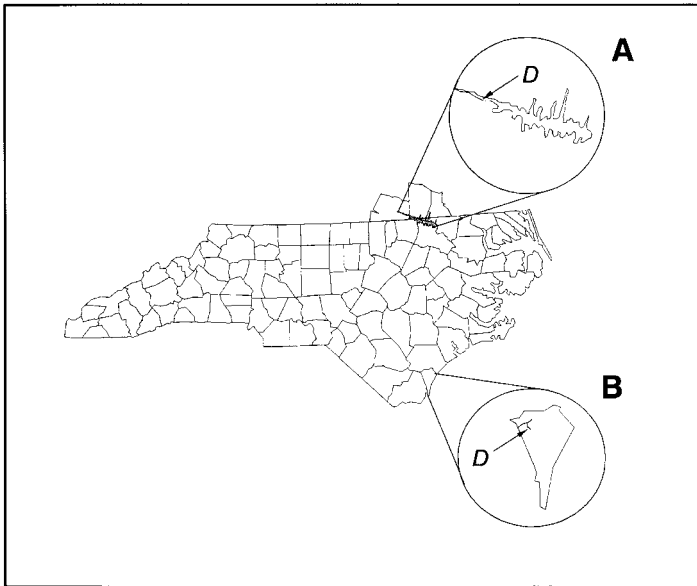


Figure 1: The location of (A) Lake Gaston and (B) Burnt Mill Creek in North Carolina. The areas of each body of water infested with dioecious hydrilla are marked (D).

1993. Leaf lengths and internode distances were measured at the termination of the experiment, after 122 days growth. Data were analyzed by analysis of variance and Duncan's multiple range test was used to compare means.

Electrophoretic and RAPD assays for biotype were conducted at the USDA ARS Aquatic Weed Laboratory, University of California, Davis. For non-denaturing gel electrophoresis, tubers were ground in 50 mM Tris Cl, pH 7.8, with 10% (v/v) glycerol and 20 mM 2-mercaptoethanol. Debris was removed by a brief centrifugation at 10,000 rpm in a Beckman Microfuge E and a 20µl sample of the supernatant solution from each extract was analyzed by electrophoresis. Electrophoresis on a 7.5% total acrylamide gel was under non-denaturing conditions, as described (Ryan 1988): protein was stained with Coomassie Brilliant Blue R. Extracts were analyzed from two tubers from each of the 3 accessions from North Carolina and from the known dioecious plants from Florida. Additional controls were run: one pair of tubers was from dioecious plants from Marion County, FL. Additional tubers of monoecious hydrilla were from plants collected in Lake Anne, NC, maintained at the USDA Aquatic Weed Laboratory in Davis since 1988.

Genetic identity of the different hydrilla accessions was further confirmed using the random amplified polymorphic DNA (RAPD) assay (Williams et al. 1990). In this assay, a decameric oligonucleotide primer is used to promote amplification of relatively short regions of DNA by the enzyme DNA polymerase. Products are separated according to size by electrophoresis on an agarose gel. Gels are treated with dilute solutions of ethidium bromide and the amplified DNA-dye complexes are visualized by their fluorescence under ultra-violet light.

For this work, DNA was isolated by the method of Saghai-Marooof et al. (1984) from plants grown from tubers in sterilized distilled water for 15 to 21 days. DNA was quanti-

fied by absorbance at 260 nm, and RAPD reactions were run using 15 nanograms of DNA in 25 µl total reaction volume. Other components were 10 mM Tris Cl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.001% (w/v) gelatin and 200 mM each dATP, dCTP, dGTP, and dTTP. The oligonucleotide primer was added to a final concentration of 0.2 µM. All components of the reaction were mixed in a 500 µl tube and the mixture was overlain with 35 µl mineral oil. Reaction mixtures were placed in the block of a Perkin-Elmer Cetus thermocycler and brought to 94 C for two minutes. The DNA polymerase (Taq polymerase from Perkin-Elmer Cetus⁴, Norwalk, CT) was diluted with a buffer of 10 mM Tris Cl, 1 mM sodium ethylenediaminetetraacetate, pH 7.8, immediately before use; 1 unit of enzyme activity in 1 µl of buffer was added to each tube at 94 C to initiate the reaction. The amplification was run for 40 cycles of 30 secs at 94 C, 30 sec at 37 C, and 1 min at 72 C. Reaction products were separated by electrophoresis on a 1.5% (w/v) agarose gel in 89 mM Tris base, 89 mM boric acid, 1 mM sodium ethylenediaminetetraacetate. Molecular weight standards were run along with the amplification reactions. From the standards, an equation for a line was generated by a linear regression of the logarithm of the molecular weight as a function of distance travelled from the origin. This equation was used to assign the molecular weights, in base-pairs, of the amplification products. Primer G17 from Operon Technologies, Inc., Emeryville, CA, was used since it distinguishes the two biotypes presently found in the U.S (data not shown). All buffers and reagents for the RAPD reaction were from Perkin-Elmer Cetus. Determination of informative primers for hydrilla will be reported elsewhere.

DNA was isolated as above from sprouting hydrilla tubers from other sources. For the RAPD assay, tubers from plants originally from Trapp Pond, DE, Dyke Marsh, VA and the Potomac River were provided by Dr. Kerry Steward, USDA ARS Aquatic Weed Laboratory, Ft. Lauderdale, FL. Tubers from Marion County, FL, were provided by Dr. Gary Buckingham, USDA ARS, Gainesville, FL, while tubers from Newside Lateral, Imperial County, CA were provided by Mike Mizumoto, Imperial Irrigation District, Brawley, CA. Two tubers from each collection site were used.

RESULTS AND DISCUSSION

On the Virginia side of Lake Gaston, plants of the putative dioecious biotype were found growing in close proximity to monoecious plants. In Figure 2, the appearance of leaves and stems from plants of the two biotypes are compared in a photo taken in the field at the time of collection. The putative dioecious plant had shorter internode distances, longer leaves, and was darker green in appearance than the monoecious plant.

In experiments in the phytotron, female flowers were observed on the suspected dioecious plants from Burnt Mill Creek and Lake Gaston 96 days after the beginning of the experiment (July 25, 1993). The known dioecious plants from Florida produced female flowers by this time as well. The monoecious plants from Lake Gaston produced female flowers by this date, and male flowers 106 days after the beginning of the experiment (August 4, 1993). Both the

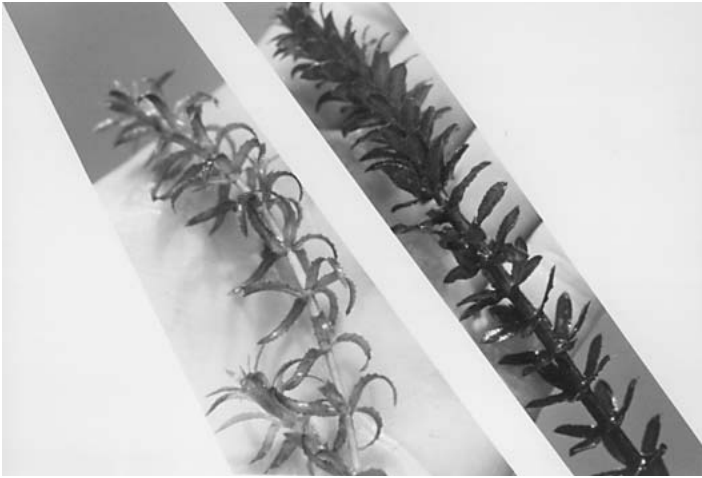


Figure 2. Leaves and stems of monoecious (left) and dioecious hydrilla (right), collected in the same area of Lake Gaston.

known and suspected dioecious plants had longer leaves and shorter internodes than monoecious plants grown under the same conditions. Values for the internode distances and leaf lengths are shown in Table 1. The suspected dioecious plants from Lake Gaston and Burnt Mill Creek had internode distances which were similar to those of the known dioecious

plants from Florida and different from that of the monoecious plant from Lake Gaston. Likewise, the two suspected dioecious plants from North Carolina had leaves of greater length than either the known dioecious plant from Florida or the monoecious plant from Lake Gaston. Both known and suspected dioecious plants produced a dense canopy at the water surface, while monoecious hydrilla first spread at the surface of the hydrosol and then grew upward. These observations are in accord with those of Van (1989) on the growth habit of plants of the two biotypes. All plants in this experiment produced small numbers of tubers. Those from the monoecious plants from Lake Gaston were brown, had pronounced leaf scales and were 10 ± 0.2 mm long (mean \pm s.d., N=24). Tubers of both known and suspected dioecious plants were white and smooth. Those from suspected dioecious plants from Lake Gaston were 16 ± 0.3 mm long (mean \pm s.d., N=6) and those from Burnt Mill Creek were 18 ± 0.2 mm long (mean \pm s.d., N=8). Those from dioecious plants from Florida were 14 ± 0.2 mm long (mean \pm s.d., N=21). The tuber lengths suggest that suspected dioecious plants from Lake Gaston and Burnt Mill Creek are different from the monoecious plants from Lake Gaston. It has been established that the mean weights of tubers from dioecious plants are greater than those of monoecious plants grown under similar conditions (Spencer et al. 1987) and this suggests that the larger tubers in this experiment were from dioecious

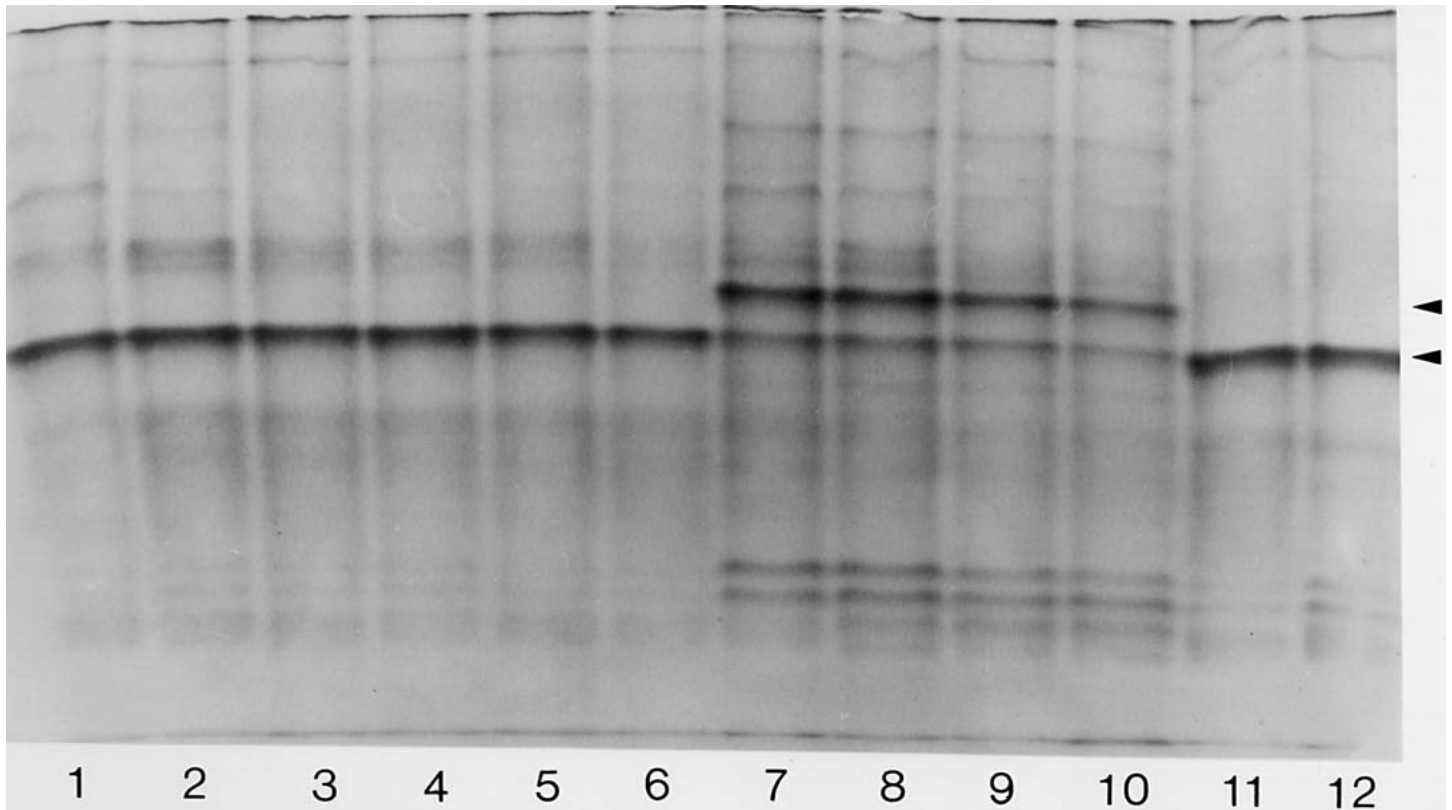


Figure 3. Extracts of hydrilla tubers after non-denaturing electrophoresis, stained for protein. Sources of the tubers were: Lanes 1 and 2, Burnt Mill Creek, NC; Lanes 3 and 4, known dioecious plants from Florida; Lanes 5 and 6, suspected dioecious hydrilla, Lake Gaston; Lanes 7 and 8, monoecious hydrilla, Lake Gaston; Lanes 9 and 10, monoecious hydrilla from the USDA Aquatic Weed Lab, Davis, CA; Lane 11 and 12, dioecious hydrilla from Marion County, FL.

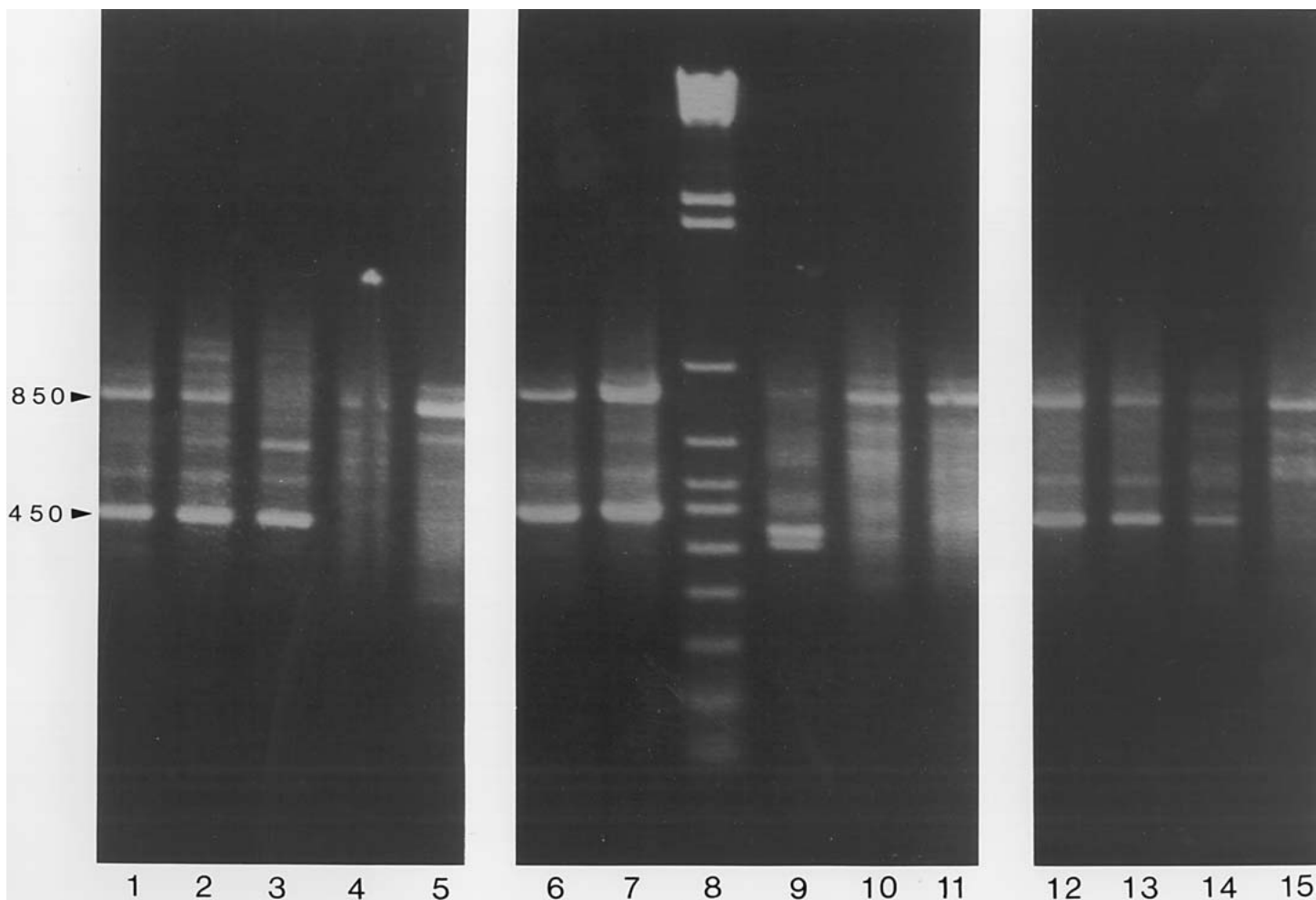


Figure 4. RAPD reaction between primer G17 and various DNA's.: Lane 1, Lake Gaston, presumptive dioecious; Lanes 2 and 3, two individuals from Marion County, Florida (dioecious); Lanes 4 and 5, two individuals from Trapp Pond, DE (monoecious), Lanes 6 and 7, two individuals from the Florida culture from North Carolina (dioecious); Lane 8, molecular weight standards; Lane 9, Dyke Marsh, VA (monoecious); Lanes 10 and 11, two individuals from Lake Gaston (monoecious), Lanes 12 and 13, two individuals from Newsider lateral, Imperial County, CA (dioecious); Lane 14, Burnt Mill Creek, NC; Lane 15, Potomac River (monoecious). The reactions were run simultaneously.

plants. The developmental stage of the tubers in this experiment is not known, however. This prevents a meaningful comparison of tuber size in this experiment with size determined from field collections at the end of the growing season, when a preponderance of mature tubers is expected.

A non-denaturing electrophoretogram of tuber extracts stained for protein is shown in Figure 3. Extracts of known

TABLE 1. INTERNODE DISTANCES AND LEAF LENGTHS OF HYDRILLA IN CULTURE. VALUES ARE MEANS AND STANDARD DEVIATIONS, N=10.

| Source | Internode distance, mm | Leaf length, mm |
|-------------------------|------------------------|-----------------|
| Lake Gaston, monoecious | 16.1 (1.45) A | 12.0 (2.05) C |
| Burnt Mill Creek | 12.0 (2.05) B | 16.1 (1.45) A |
| Lake Gaston, dioecious | 11.0 (0.82) B | 16.1 (1.45) A |
| Florida | 11.0 (0.82) B | 14.0 (0.82) B |

Means in a column followed by the same letter are not significantly different according to Duncan's Multiple Range procedure ($\alpha=0.05$)

dioecious hydrilla tubers from two sites in Florida in lanes 3, 4, 11 and 12 show a single major band, noted by the arrow at the left side of the figure. Extracts of suspected dioecious tubers from Burnt Mill Creek (lanes 1 and 2) and Lake Gaston (lanes 5 and 6) show this same pattern. Extracts of monoecious tubers maintained in culture in Davis, CA, but originally from Lake Anne, NC (lanes 9 and 10) show two bands in this region, as do extracts from tubers of monoecious plants from Lake Gaston (lanes 7 and 8).

The results from the RAPD assay support the assessment of biotype from electrophoresis of tuber proteins. As shown in Figure 4, in the RAPD reaction with Primer G17, DNA from dioecious plants had two major amplification products, at approximately 850 and 450 base-pairs. DNA from monoecious plants had only a single amplification product, of molecular weight 850 base-pairs. Some additional bands are produced, e.g. in lane 3 there is a strong amplification product between the two marked bands. This is not interpreted further. Also, in lane 9, a strong amplification product is seen

with molecular weight less than 450 base-pairs. Again, this pattern is not interpretable.

Results of growth studies, analysis of tuber proteins by electrophoresis, and RAPD analysis confirmed the presence of both hydrilla biotypes in Lake Gaston. In addition, available evidence indicated that the dioecious biotype of hydrilla was present in Burnt Mill Creek.

The discovery of both biotypes of hydrilla at Lake Gaston is the first report of their coexistence in the same body of water in the U.S. Lake Gaston lies on the NC-VA border at latitude 36°30' N. The present northernmost infestation of hydrilla known in the U. S. is at Redding, CA, at 40°35' N. Spencer and Anderson (1986) suggest that the ranges of both biotypes may extend even further north, based on consideration of climate and day length. The discovery of the coexistence of the two biotypes in Lake Gaston poses concerns for management strategy. Plants of the dioecious biotype were observed to be rooted and intact throughout the winter of 1992-93 and 1993-94 (C. R. Coley, unpublished observation) and thus well established. The monoecious plant in the Potomac River has been described as an annual (Sutton et al. 1992). Steward (1993) has reported that viable seeds are produced from crosses between the U.S. female dioecious plant and pollen from the U.S. monoecious plant. It remains to be demonstrated that flowering of both biotypes is synchronous under field conditions, although it was in the phytotron. Furthermore the production of viable seed from the interaction of these plants may require an extended period of seed development (Lal and Gopal 1993) and be unlikely under the conditions found in northern North Carolina. Even if seed production is unlikely, it must still be considered a possibility.

The discovery of a plant of the dioecious biotype at Burnt Mill Creek in southeastern North Carolina presents other concerns. Additional spread of the plant in this region due to human activities, by waterfowl, or through the action of wind and water during severe storms are possibilities. Further research is needed to determine environmental and physical conditions required by plants of the two biotypes, especially with regard to the hydrosols in the diverse littoral zones of lakes, reservoirs and rivers of the eastern seaboard.

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