J. Aquat. Plant Manage. 35: 10-14.

Hydrilla verticillata (Hydrocharitaceae) in Connecticut

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ABSTRACT

A specimen of hydrilla (*Hydrilla verticillata* (L.f.) Royle) collected at Mystic, Connecticut in 1989 was recently discovered in the University of Connecticut herbarium. Unnoticed previously because of its misidentification as egeria (*Egeria densa* Planch.), this specimen is the first authenticated record of hydrilla in New England, and represents the most northern locality of the species currently known in eastern North America. A 1996 field survey verified that hydrilla continues to thrive at the Connecticut site. Connecticut plants were positively identified as hydrilla by morphological features, and by comparing the *rbc*L gene sequence of Connecticut specimens with a hydrilla plant from India. Internode lengths of Connecticut hydrilla exceeded those reported for both dioecious and monoecious strains grown in greenhouse conditions. However, leaf lengths of Connecticut hydrilla were comparable to those of the dioecious strain designated as 'USA hydrilla I'. A RAPD profile of Connecticut hydrilla produced the molecular marker that reportedly distinguishes the dioecious strain. Cytological analysis indicated that the Connecticut hydrilla plants are triploid (2n = 3x = 24). Hydrilla in Connecticut presumably represents an introduction of dioecious plants. Hydrilla grew well on both sandy and mucky substrates and apparently overwinters in Connecticut by production of numerous, subterranean stem tubers.

Key words: chromosome number, distribution, identification, North America, *rbc*L sequence, RAPD, triploid, weed.

INTRODUCTION

Hydrilla verticillata (hydrilla) is an Old World species that was first introduced to North America in Florida during the 1950's (Blackburn et al. 1969, Haller 1982, Weldon et al. 1969). Hydrilla is an extremely invasive species and by 1967, it had spread throughout Florida (Blackburn et al. 1969) and subsequently to Alabama, California, Colorado, Delaware, the District of Columbia, Florida, Georgia, Iowa, Louisiana, Maryland, North Carolina, South Carolina, Texas, Virginia, Washington and as far south as Tamaulipas, Mexico (Haller 1982, C. B. Hellquist pers. comm., Novelo & Martínez 1987, J. Parsons pers. comm., Ryan et al. 1995, Steward et al. 1984, Trudeau 1982). Only the populations in Iowa have reportedly been eradicated (Haller 1982).

Before 1980, hydrilla was not known to occur in Connecticut or other parts of New England (Countryman 1970, Dowhan 1979). Trudeau (1982, p. 53) conveyed reports of "minor" hydrilla infestations in Connecticut which, ". . . could not be confirmed with state officials." Rumors of hydrilla in Connecticut had reached regional aquatic botanists (Hellquist pers. comm.), but remained unverified. We recently discovered a specimen of hydrilla in the University of Connecticut herbarium which had been misidentified and misfiled as *Egeria densa* [*Hendrickson s.n.*, 14 Oct 1989

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(CONN)]. This specimen was collected from a pond in Mystic, Connecticut and is the first authenticated record of hydrilla in New England. Our field surveys in spring, 1996 confirmed that hydrilla continues to thrive at this site. Prior to this report, the 1976 discovery of hydrilla in Delaware (Steward et al. 1984) represented the farthest northeastern extension of hydrilla in North America. This report summarizes the significance of the Connecticut population of hydrilla which, to our knowledge, currently represents the northeasternmost locality of the species in North America.

MATERIALS AND METHODS

We verified the identification of Connecticut specimens by the presence of several features that distinguish *Hydrilla* from the morphologically similar genera *Elodea* and *Egeria* (Aulbach-Smith 1990, Blackburn et al. 1969, Cook 1990). These included: 1) leaves in whorls of 3-8 [typically 3 in *Elodea*; 3-6 in *Egeria*]; 2) sharply toothed leaf margins [serrulate in *Elodea* and *Egeria*]; 3) fringed squamulae intravaginales [entire in *Elodea* and *Egeria*]; and 4) subterranean stem tubers [absent in *Elodea* and *Egeria*]. Connecticut specimens lack the abaxial midrib teeth that occur on some hydrilla specimens. We sequenced 1183 nucleotides of the *rbc*L gene of Connecticut hydrilla following methods reported previously by Les et al. (1993). The DNA sequence was compared to that obtained from a hydrilla plant from India sent to us and identified by C. D. K. Cook in Zürich, Switzerland. The *rbc*L sequence of the Old World hydrilla was obtained in the same fashion. The *rbc*L sequences of other Hydrocharitaceae (e.g. *Elodea*, *Egeria*, *Lagarosiphon*) are readily distinguishable from hydrilla (Les et al. in press). RAPD analyses (see below) provided yet additional corroboration of our identification.

Means and standard deviations were calculated for the lengths of 25 internodes and 25 leaves located near the middle of shoots from Connecticut plants. These were compared with published data used to distinguish between the 'USA hydrilla I' and 'USA hydrilla II' biotypes of hydrilla (Ryan et al. 1995). Mitotic chromosome preparations for Connecticut hydrilla plants were obtained following the methods reported by Langeland (1989).

We obtained a random amplified polymorphic DNA (RAPD) profile for the Connecticut hydrilla following the methods reported by Ryan et al. (1995) which uses the'G17' primer sequence (Operon Technologies, Inc., Emeryville, CA) to putatively distinguish the same two biotypes. We necessarily modified the procedure reported by Ryan et al. (1995) which specified adding dNTP's as "200 mM each". Because stock dNTP solutions are typically furnished as only 10 mM each dNTP, we assumed that Ryan et al. (1995) inadvertently reported mM rather than µM quantities. We routinely perform RAPD analyses using 100 µM of each dNTP (25 µl reactions), but adjusted the dNTP's to 200µM each to simulate the amplification conditions used by Ryan et al. (1995). The methods reported by Ryan et al. (1995) did not identify the molecular weight marker used in their analyses. We calculated our RAPD fragment sizes using a *Bst*E II digest of lambda DNA (New England Biolabs, Beverly, MA) as the reference marker.

Sediments from the hydrilla pond were sampled from four locations that appeared to represent extremes from sandy to mucky texture at the site. The percentage of organic matter (%OM) was determined for these samples following the methods reported in Ball (1964).

RESULTS AND DISCUSSION

Morphological features readily identified the Connecticut plants as hydrilla. We confirmed the identification by comparing an 1183 nucleotide region of the *rbc*L gene obtained from a known specimen of hydrilla from India, with that of specimens obtained from the Mystic population. The sequences matched exactly.

We obtained a chromosome count of 2n = (3x) = 24 for the Connecticut hydrilla plants, confirming that they are triploids. The relatively large chromosomes of hydrilla were evenly spread in several of the preparations which allowed for unambiguous counting.

As many as 24 electrophoretically determined 'biotypes' of hydrilla have been identified worldwide and two of these reportedly occur in the United States (Verkleij and Pieterse 1991). A female strain of dioecious hydrilla ('USA hydrilla I') is believed to represent the first introduction into the USA which occurred sometime before 1960; a monoecious biotype ('USA hydrilla II') is thought to have been introduced secondarily around 1980 (Vandiver et al. 1982, Verkleij and Pieterse 1991).

Monoecious and dioecious hydrilla grown under greenhouse conditions have been distinguished by differences in leaf and internode length; monoecious plants produced shorter leaves (12.0 ± 2.05 mm) and longer internodes (16.1 ± 1.45 mm) than dioecious plants (14.0 - 16.1 ± 0.82 - 1.45 mm and 11.0 - 12.0 ± 0.82 - 2.05 mm respectively) (Ryan et al. 1995). Field-collected plants of hydrilla from Connecticut had leaf lengths (14.5 ± 1.39 mm) that were comparable to those of dioecious plants grown in the greenhouse and internode lengths (19.7 ± 3.64 mm) that exceeded those of monoecious plants grown in the greenhouse. However, our comparisons between greenhouse grown and field plants must be interpreted conservatively, and do not establish conclusively whether Connecticut plants represent the monoecious or dioecious strain.

We endeavored to resolve this question by comparing the RAPD profile (G17 primer) of Connecticut plants with those reported by Ryan et al. (1995). Genetic markers such as those obtained by the RAPD method provide greater confidence than morphological traits such as leaf and internode lengths which are susceptible to environmentally induced plasticity. With necessary modifications to the RAPD protocol (see Materials and Methods), we obtained a RAPD profile for Connecticut hydrilla plants that perfectly matched the result reported by Ryan et al. (1995) for dioecious hydrilla plants surveyed from California, Florida and North Carolina.

Connecticut plants (Figure 1) possessed two major G17 primer amplification products of approximately 880 and 400 b.p. in size (estimates of fragment size are somewhat variable). These sizes correspond closely to the estimated 850 and 450 b.p. bands identified as the major diagnostic components of the G17 RAPD profile for dioecious hydrilla (Ryan et al. 1995). Monoecious hydrilla plants surveyed from Delaware, Virginia and the Potomac (geographically, the closest hydrilla populations to Connecticut) reportedly lack the 450

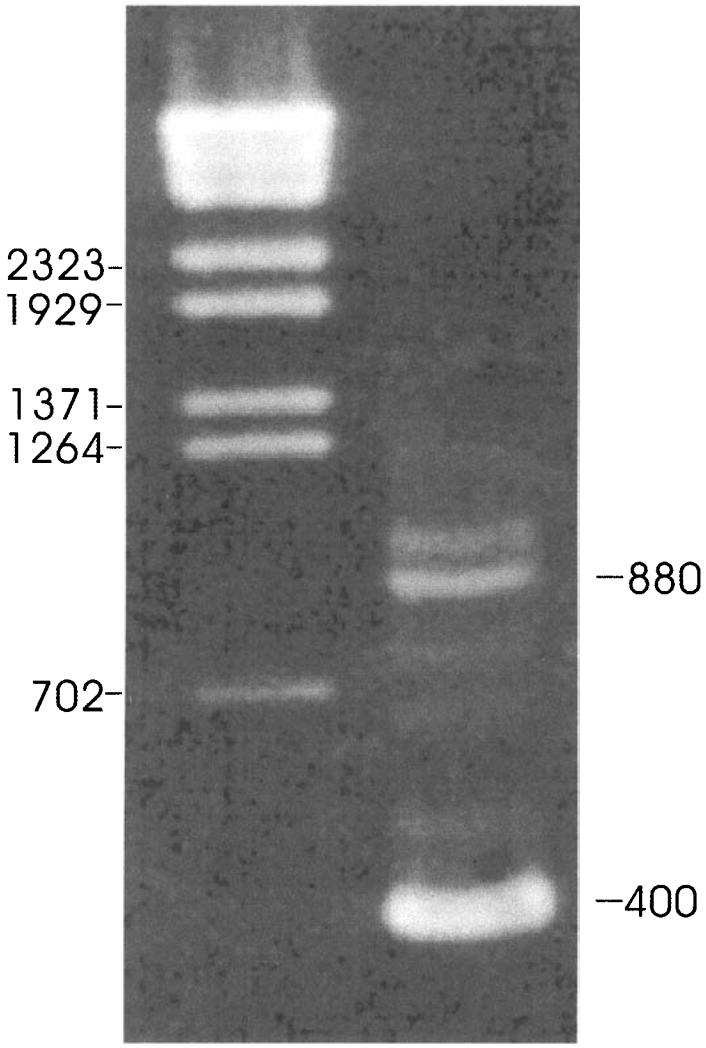

Figure 1. RAPD profile of Connecticut Hydrilla plants (right lane) using a primer (Operon, G17 sequence) that putatively distinguishes monoecious and dioecious strains in the United States. Our results reproduced both the approximately 450 and 850 b.p. bands that Ryan et al. (1995) used to identify the dioecious strain. The slight differences in our estimated sizes for the RAPD marker bands is insignificant. The RAPD profile of Connecticut plants also shows three fainter bands that appear in between these markers, and a faint band of a slightly higher molecular weight; these faint bands are also evident in some of the dioecious RAPD profiles reported by Ryan et al. (1995). The molecular weight marker (left lane) is a *Bst*E II digest of lambda DNA.

b.p. band (Ryan et al. 1995) which we obtained as a major amplification product for Connecticut plants. Thus, results from RAPD analysis indicate that Connecticut hydrilla represents the dioecious strain, or at least that the Connecticut plants were not derived from the nearest hydrilla populations which have been determined as monoecious.

Dioecious and monoecious biotypes of hydrilla in North America were originally thought to be distinguishable cytologically. Although the 'USA hydrilla I' biotype was presumed to be exclusively triploid (Verkleij et al. 1983), tetraploid individuals have been reported from Alabama (Davenport 1980). The monoecious 'USA hydrilla II' strain is often regarded as exclusively diploid (Verkleij et al. 1983), but Harlan et al. (1984) and Langeland (1989) subsequently obtained triploid counts from all monoecious hydrilla plants they examined, including those from populations reported previously to be diploid by Verkleij and Pieterse (1986). Similarly, all of the 30 monoecious hydrilla populations examined from Japan were also triploid (Nakamura and Kadono 1993). The extent of diploid hydrilla populations in the USA remains ambiguous. Verkleij and Pieterse (1991, pp. 50-51) characterized several collections of hydrilla from North Carolina as having chromosome numbers of 16/24 or 24, yet added a footnote indicating that there was "no evidence for correct chromosome number" in those cases. We cannot understand why these numbers would even be tentatively assigned in the absence of evidence for the counts. This leads us to conclude that at least some of the reports of diploid hydrilla in the USA may be erroneous. Our triploid chromosome counts for the Connecticut hydrilla population is consistent with the introduction of the dioecious strain, but is not conclusive given that others have reported the monoecious strain to possess a triploid complement.

Langeland (1989) suggested that endopolyploidy may explain the different cytotypes of hydrilla. Certainly, the discovery of tetraploid hydrilla plants (Davenport 1980) is consistent with this hypothesis. Endopolyploidy occurs extensively in clonal aquatic angiosperms (Les and Philbrick 1993). The prospect of endopolyploidy raises the possibility that some of the variation observed in North American hydrilla may reflect somatic chromosomal mutations in the original triploid female stock. Researchers examining genetic differences in hydrilla using isozymes and RAPD markers should be aware that differences in ploidy might also generate variability in isozyme and RAPD profiles. Variation in isozyme and RAPD profiles due to genic somatic mutations have been documented in other clonal aquatic species (Les and Gabel 1996). Accordingly, different genotypes of hydrilla do not invariably indicate new or separate introductions of the species, but may also result from somatic mutational processes.

Dioecious hydrilla strains in other parts of the world appear to be more widely distributed in temperate regions with monoecious strains more common in warmer regions (Cook and Luond 1982, Nakamura and Kadono 1993). However, the North American monoecious strain apparently is adapted to the shorter growing seasons of northern habitats (Verkleij and Pieterse 1991). Initially, hydrilla was not regarded as a problem species in northern North America because it was considered to be incapable physiologically of survival in colder climates (Trudeau 1982). However, the spread of both monoecious and dioecious strains into northern latitudes has now become a serious threat (Ryan et al. 1995). Our evidence that Connecticut plants probably represent dioecious hydrilla further cautions against underestimating the potential spread of this species into temperate climates. Steward et al. (1984) suggested that hydrilla could potentially grow throughout the United States and much of Canada, and was expanding its range northward. This warning was reiterated by Balciunas and Chen (1993) who emphasized that hydrilla extends to far northern latitudes in Asia where it occurs to within 9° latitude of the Arctic Circle. From similar reasoning, Langeland (1996) contemplated whether hydrilla would eventually become as problematic in

TABLE 1. VARIOUS NORTHERLY DISTRIBUTIONS (APPROXIMATE) OF *HYDRILLA VERTICILLATA* WORLDWIDE. MYSTIC, CONNECTICUT IS PRESENTLY THE NORTHERNMOST STATION OF HYDRILLA KNOWN IN EASTERN NORTH AMERICA, BUT THIS LATITUDE HAS BEEN GREATLY SURPASSED BY HYDRILLA IN WASHINGTON STATE AND SEVERAL OTHER COUNTRIES.

Locality	Country	Latitude	Reference
Irkutsk	Russia	58°30'N	Balciunas & Chen 1993
Eastern Europe	Latvia	57°30'N	Fedchenko 1934
Novosibirsk, Siberia	Russia	55°N	C. B. Hellquist, pers. comm. 1996
Stettin	Poland	53°N	Steward et al. 1984
Galway County	Ireland	53°N	Steward et al. 1984
Harbin	China	46°N	Balciunas & Chen 1993
Hokkaido	Japan	43°N	Nakamura & Kadono 1993
King Co., Washington	USA	47°21'N	J. Parsons, pers. comm. 1996
Mystic, Connecticut	USA	41°20'N	This study
Redding, California	USA	40°35'N	Ryan et al. 1995
Lee County, Iowa	USA	40°30'N	Trudeau 1982
Sussex Co., Delaware	USA	39°N	Steward et al. 1984
Washington, D.C.	USA	39°N	Steward et al. 1984

northern states as it is in the south. Indeed, hydrilla occurs well north of the Connecticut population in other parts of the world (Table 1) and its presence in New England documents the continued northerly spread of the species in North America.

Survival of hydrilla at higher North American latitudes appears to be exclusively by means of stem tubers and axillary turions given that whole plants do not overwinter in northern localities (Harlan et al. 1985). Nakamura and Kadono (1993) observed that monoecious hydrilla in Japan produced only stem tubers and dioecious plants produced only axillary turions. Apparently, this distinction does not hold as well for dioecious and monoecious hydrilla in the USA which both reportedly produce tubers (Sutton et al. 1992). Monoecious hydrilla plants produce prolific numbers of stem tubers throughout the year (> 6,000/plant/season) in contrast to dioecious strains that form tubers only during the winter (Sutton et al. 1992).

Many specimens that we recently collected from the Mystic, Connecticut site consisted of plants that had sprouted from stem tubers, and no flowers were present at the time of our collection (June, 1996). The original specimen label also indicated that no sexual plants were observed at the site later in the season (October). Stem tuber production is influenced by sediment content with plants grown on marl substrates yielding an approximately five-fold greater number of tubers than plants on sandy substrates (Bruner and Batterson 1984). This factor suggests that marl habitats, which are confined to western Connecticut, may be more vulnerable to severe hydrilla outbreaks. Because hydrilla reportedly grows better on organic rather than sandy substrates (Spencer et al. 1992), many Connecticut lakes (which typically have low organic matter content) may be less vulnerable to severe infestations than other areas. However, this optimism may be unwarranted. The substrate of the Mystic, Connecticut hydrilla site ranged from sandy to mucky with low to moderate levels of organic matter. Sandy sites contained only 0.3%OM; whereas, the mucky sites ranged from 19-24%OM. Despite these differences in organic matter content, the hydrilla appeared to thrive on both sediment types. We did not further evaluate the relative productivity of hydrilla plants on different substrates.

The original specimen label stated that hydrilla at the Mystic site, ". . . densely fills the pond and is a virtual monospecific stand." Hydrilla has continued to be weedy at this site for the past several years (Anon pers. comm.). These observations warn that the Mystic, Connecticut hydrilla site should be given serious consideration with steps taken to eradicate the plants or at least minimize the probability of its spread to other areas.

We are not certain how the hydrilla plants were introduced into Connecticut. Waterfowl frequent the pond, and may have brought fragments in from other localities. Stem tubers can also be transported by waterfowl (Langeland 1996). However, this explanation would indicate that other populations of hydrilla may exist nearby. We searched all potential sites in the near vicinity of the Mystic population, but found no evidence of hydrilla elsewhere. A more comprehensive field reconnaissance is presently underway. White water lily (*Nymphaea odorata* Ait.) also occurs in the pond and we attempted to determine whether this was a natural population or had been planted for ornamental purposes. Introduction of hydrilla via contaminated nursery stock has been suggested (Steward et al. 1984) and could explain the unusual occurrence of the plants in this fairly isolated site. However, we are presently unable to determine the source of the water lilies in the Mystic hydrilla pond.

The Connecticut hydrilla site is in the midst of a heavily touristed area which receives visitors from all parts of the country including hydrilla-rich states such as Florida. Because Connecticut hydrilla appears to be a dioecious strain (which is the most common type throughout the southern USA), it is conceivable that the introduction to this site may have resulted from careless disposal of aquarium stock.

Even after three decades since its original introduction into North America, hydrilla continues to be overlooked because of taxonomic misidentifications. Hydrilla was originally misidentified as American elodea (*Elodea canadensis* Michx.) following its introduction into Florida (Blackburn et al. 1969) and Delaware (Steward et al. 1984). Our Connecticut specimen of hydrilla had gone unnoticed for seven years because it had been misidentified as *Egeria densa*. We encourage a thorough examination of herbarium specimens (e.g.,

folders of *Elodea*, *Egeria*, *Najas*), especially in other northeastern herbaria, to search for other possibly misidentified hydrilla specimens.

ACKNOWLEDGEMENTS

Leigh Knuttel and Steve Sisk provided historical information on the hydrilla pond. Phil Ficara performed the organic matter analysis and Ron Aakjar sequenced the hydrilla *rbc*L.

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