

Monitoring Hydrilla Using Two RAPD Procedures and the Nonindigenous Aquatic Species Database

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ABSTRACT

Hydrilla (*Hydrilla verticillata* (L.f.) Royle), an invasive aquatic weed, continues to spread to new regions in the United States. Two biotypes, one a female dioecious and the other monoecious have been identified. Management of the spread of hydrilla requires understanding the mechanisms of introduction and transport, an ability to map and make available information on distribution, and tools to distinguish the known U.S. biotypes as well as potential new introductions. Review of the literature and discussions with aquatic scientists and resource managers point to the aquarium and water garden plant trades as the primary past mechanism for the regional dispersal of hydrilla while local dispersal is primarily carried out by other mechanisms such as boat traffic, intentional introductions, and waterfowl. The Nonindigenous Aquatic Species (NAS) database is presented as a tool for assembling, geo-referencing, and making available information on the distribution of hydrilla. A map of the current range of dioecious and monoecious hydrilla by drainage is presented. Four hydrilla samples, taken from three discrete, non-contiguous regions (Pennsylvania, Connecticut, and Washington State) were examined using two RAPD assays. The first, generated using primer Operon G17, and capable of distinguishing the dioecious and monoecious U.S. biotypes, indicated all four samples were of the monoecious biotype. Results of the second assay using the Stoffel fragment and 5 primers, produced 111 markers, indicated that these samples do not represent new foreign introductions. The differences in the monoecious and dioecious growth habits and management are discussed.

Key words: Randomly amplified polymorphic DNA, *Hydrilla verticillata*, monoecious, dioecious, spread, dispersal.

INTRODUCTION

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is an invasive aquatic weed whose range in the Old World is vast. It extends from New Zealand and Australia through many of the Southwest Pacific Islands, the Philippines, Taiwan, and Japan (Cook and Lüönd 1982, Pieterse 1981). In Asia it extends from

South-east Asia north through China and into Siberia and west to Pakistan. It has a disjointed range in Africa and northern Europe (Cook and Lüönd 1982, Pieterse 1981). Since its introduction hydrilla has spread aggressively throughout the United States. A dioecious female biotype, first identified in 1959 (Blackburn et al. 1969) was reported to have been introduced from Sri Lanka to Florida in the early 1950s by a tropical fish and plant dealer (Schmitz et al. 1990). The current range of this plant is throughout the south with separate distributions in California (Yeo and McHenry 1977, Yeo et al. 1984). A second introduction was reported in 1976 from Delaware and from the Potomac river around 1980 (Haller 1982, Steward et al. 1984). This second biotype is monoecious and has spread across the central Atlantic states (Kay 1992, Langeland 1996). It is seemingly more adapted to areas with short growing seasons (Van 1989) and is capable of producing viable seed (Langeland and Smith 1984, Conant et al. 1984).

As hydrilla and other nonindigenous aquatic species spread, the need for resource management tools to track infestations increases. Aquatic plant monitoring programs, the most extensive and up to date sources for spatial data on nonindigenous plants, are not equally available nationwide. After polling natural resource agencies from all 50 states, Bartodziej and Ludlow (1997) reported that only seven states conduct full scale aquatic plant monitoring programs. Twenty states have partial programs, that is, they are limited in the number of waters, plant species, or years surveyed. Twenty three states are without programs. Other sources for occurrence information on nonindigenous plants may be obscure and are widely dispersed (Jacono and Boydston 1998). This study demonstrates the use of the Nonindigenous Aquatic Species (NAS) Database for cataloging and presenting spatial relationships from the available occurrence data on hydrilla.

The two hydrilla biotypes may occur simultaneously in the same water body or within the same region. Ryan et al. (1995) first reported the occurrence of the dioecious biotype in a lake with an existing monoecious population. In the absence of flowering, the two biotypes can sometimes be distinguished by their growth habit. The dioecious biotype initially grows vertically producing a dense surface mat while the monoecious initially spreads horizontally along the sediment with less vertical elongation (Van 1989). Nevertheless, hydrilla's growth habit displays a great deal of environmental plasticity. To distinguish the biotypes accurately Ryan and Holmberg (1994) and Ryan et al. (1995) used the molecular assay known as random amplified polymorphic DNA (RAPD). They displayed a single polymorphic marker, generated

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using primer Operon G17, hereafter referred to as the "G17 fingerprinting reaction", which is present in the dioecious biotype but absent in the monoecious.

While the use of a single diagnostic molecular marker provides a useful tool for distinguishing between the two U.S. biotypes, it cannot eliminate the possibility of a third, separate introduction. To answer questions of genetic similarity one must increase the number of markers. Madeira et al. (1997) used a RAPD procedure to examine the phenetic relationships among 44 accessions of hydrilla from various regions of the world, generating 85 polymorphic markers for the analysis. Dioecious samples from Florida, Texas, and California clustered closely before joining a cluster from the Indian subcontinent. Eight monoecious samples from North Carolina, Maryland, Virginia and Delaware clustered together along with an accession from Seoul, Korea. These data reinforced the widely held impression that there have been two foreign introductions. However, when domestic introductions appear in regions widely separated geographically from the nearest infestation, it seems relevant to re-investigate whether they represent foreign introductions or further dispersion by one of the two existing biotypes.

The second goal of this paper, therefore, is to demonstrate the utility of molecular techniques in answering questions of biotype or origin using several geographically isolated samples. The G17 fingerprinting reaction used to distinguish U.S. biotypes is repeated and followed by a more detailed examination using the Madeira et al. (1997) multi-primer phenetic analysis to determine whether samples might represent new foreign introductions. Three infestations (four samples), characterized by geographical separation from established distributions, were selected for examination.

MATERIALS AND METHODS

Plant material. The first sample, collected in 1996 by the senior author from the Schuylkill River (39°58'48"N, 75°11'52"W) was, at the time this study began, both the first report of hydrilla in Pennsylvania, and the northernmost continuation of the existing range of the monoecious biotype known for the central Atlantic states. The second sample was provided by Kathy Hamel (Washington State Department of Ecology) and Ernie Marquez (Resource Management) from the Lucerne/Pipe Lakes complex, Washington State (47°22'05"N, 122°02'57"W), the most northern distribution of hydrilla in the nation and the infestation most distant from other known infestations. It was determined as monoecious previously by the G17 fingerprinting reaction conducted at the USDA-ARS Davis Laboratory (Kathy Hamel, Washington State Department of Ecology, pers. comm.). The third sample came from the Mystic, Connecticut pond (41°21'40"N, 71°58'1"W) described by Les et al. (1997) and represented the most northern and discontinuous infestation known in the Atlantic Basin at the time of the study, as well as the northernmost putative dioecious infestation. The fourth sample came from a lake at Mason Island, Connecticut (41°19'58"N, 71°58'3"W), approximately 5 km. from the Mystic site. Both Connecticut samples were provided by Nancy Balcom, Sea Grant Marine Advisory Program, University of Connecticut. The Mystic site had been treated with herbicide, and the specimen arrived looking chlorotic with

some necrosis, and with a heavy epiphytic crust (*Aufwuchs*) of algae and bacteria. Those from Mason Island arrived in healthy condition. To serve as controls, dioecious hydrilla from Florida and California and monoecious hydrilla from North Carolina and Delaware were selected from the USDA collection along with "nearest (foreign) neighbor" plants from Bangalore, India and Seoul, Korea. "Nearest neighbor" plants were selected because they were the most similar genetically to the biotypes presently found in the U.S. according to the RAPD analysis of Madeira et al. (1997). While the Bangalore plant served as an outlier to (clustered immediately outside of) the U.S. dioecious accessions, the Seoul plant integrated into (clustered with) the U.S. monoecious samples. Therefore a sample from Rawa Pening, Indonesia, one of the closest related accessions to the U.S. monoecious samples in the previous study (aside from Seoul) was included as an outlier. Apical stem fragments from each collection were thoroughly rinsed in a jet of deionized water to remove as much epiphytic material as possible, then blotted dry. Next, approximately 50 mg total wet weight of leaves was placed into a 1.5 ml microcentrifuge tube and stored in an ultracold freezer (-80C) until removed for DNA extraction.

DNA extraction, RAPD Amplifications and Gel Electrophoresis. Total DNA was extracted using the CTAB method of Doyle and Doyle (1990) as "micro" modified by Van and Madeira (1998). The DNA solution was quantified using fluorometry and stored at 4C. Two different RAPD reactions were used. The first is the G17 fingerprinting reaction used by Ryan et al. (1995) to distinguish the U.S. monoecious and dioecious biotypes with the dNTP modification noted by Les et al. (1997). The second, multiprimer reaction used AmpliTaq DNA Polymerase, Stoffel fragment (Perkin Elmer[®]), the same five primers (Operon Technologies: A4, A6, B20, G11, G14), and the reaction conditions used by Madeira et al. (1997) to analyze a world-wide collection of hydrilla. This reaction was used for the phenetic analysis. Amplifications were replicated at least three times. Amplification products were electrophoresed on 2% agarose (Sigma) gels, visualized by ethidium bromide staining, and photographed using a FO-TO/Analyst Minivisionary System (Fotodyne, Inc.).

Phenetic Analysis. Loci were scored for the presence (1) or absence (0) of bands and characterized by the primer used followed by the size in base pairs. Size was determined by reference to 100 bp DNA ladder (Gibco BRL, Bethesda, MD) spaced at regular intervals throughout the gel. A Dice similarity matrix and a corresponding UPGMA (unweighted pair-group method, arithmetic average) phenogram was generated using the NTSYS (v 1.8) program (Rohlf 1993) A Bootstrap Analysis (Felsenstein 1985) was applied to the UPGMA phenogram using the RAPDBOOT¹ program.

Distribution. The Nonindigenous Aquatic Species (NAS) database of the United States Geological Survey (USGS) is a

¹Mention of a trademark name, proprietary product, or specific equipment does not constitute a warranty by the U.S. Department of Agriculture or the U.S. Department of the Interior, and does not imply its approval to the exclusion of other products that also may be suitable.

²Black IV, W. C. 1995. FORTRAN programs for the analysis of RAPD-PCR markers in populations. Colorado State University, Ft Collins, CO 80523.

national repository for geographic accounts of all aquatic organisms introduced outside of their natural range within the U.S. Spatial information from monitoring programs, herbarium specimens, literature, professional communications and other records is assembled, evaluated and geographically referenced prior to storage in the database. An internet web site³ reporting form is available as a contact mechanism to provide potential leads on previously undocumented occurrences; these reports are confirmed by specimen submission or on-site evaluation. The database is composed of fields such as state, locality, year reported, status and method of introduction. Spatial data is geographically referenced to the USGS 8-digit Hydrologic Unit Code (HUC), a classification of river drainages, to demonstrate occurrence according to natural drainage basin. The database is used to produce dynamic distribution maps. Internet access⁵ to the data set currently provides species lists according to state or hydrologic unit selected by the user.

RESULTS AND DISCUSSION

RAPD Analysis. The G17 fingerprinting reaction is diagnostic for separating the U.S. dioecious and monoecious biotypes (Figure 1). Known monoecious plants are arrayed on the left and known dioecious on the right. Note that more bands appear in these reactions than in previous papers. This is not unusual as RAPD procedures are notoriously difficult to repeat, especially between laboratories, emphasizing the need for controls within each study. Nevertheless, a monomorphic band appears at about 850 bp as described by Ryan and Holmberg (1994) and Ryan et al. (1995). This band was placed by Les et al. (1997) at about 880 bp length. A second and polymorphic band appears at about 495 bp, appearing only in the two dioecious controls. Note that this

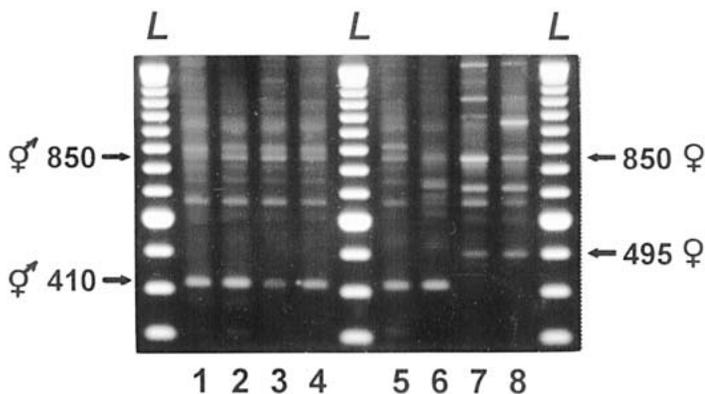


Figure 1. Results of the RAPD/ Operon G17 reaction diagnostic for the U.S. dioecious and monoecious biotypes. Known monoecious plants are North Carolina (lane 1) and Delaware (lane 2). Known dioecious plants are Florida (lane 7) and California (lane 8). Unknowns are arranged between the monoecious and dioecious and include Washington State (lane 3), Pennsylvania (lane 4), Mason Island, Ct. (lane 5), and Mystic, Ct. (lane 6). A bright marker diagnostic for monoecious appears at about 410 bp and a second moderate marker diagnostic for dioecious appears at about 495 bp.

⁵The Nonindigenous Aquatic Species (NAS) database web site can be accessed at <http://nas.er.usgs.gov>

marker does not appear in any of the four experimental accessions under examination. A dioecious marker was reported at 485 bp in Ryan and Holmberg (1994), but at 450 bp in Ryan et al. (1995). Les et al. (1997) reported a marker for the Mystic sample appearing at 400 bp. They interpreted this marker as equivalent to the 450 bp dioecious marker but did not include monoecious or dioecious controls. Our reactions show an additional polymorphic marker at about a 410 bp length, appearing in the monoecious controls, as well as in all four experimental samples. We believe this 410 bp band corresponds to the Les et al. (1997) 400 bp band suggesting that the Pennsylvania, Washington, and both Connecticut samples are monoecious (if the possibility of a third biotype introduction is discounted). Additionally we utilized a regularly spaced, 100 bp interval ladder to determine the approximate lengths of bands, while the previous studies used ladders of restriction digests which are not as regularly spaced and require greater extrapolation to estimate band length. This may account for the differences in the estimates of nucleotide (bp) length among these studies.

The five primer (Madeira et al. 1997) RAPD analysis (example gels not shown) produced a total of 111 consistent polymorphic markers (A4-22, A6-26, G14-21, B20-20, G11-22). Figure 2 displays the UPGMA (unweighted pair-group method, arithmetic average) phenogram generated from Dice Similarities. The dioecious controls (Florida and California) cluster together tightly and then are joined by the Bangalore accession, which was the "nearest neighbor" to the dioecious U.S. plants in Madeira et al. (1997). Note also that the two monoecious controls (Delaware and North Carolina) and the Korean (probable origin) plant cluster with the four experimental biotypes. The percentages in Figure 2 appearing to the right of each branch are the bootstrap for that cluster. High bootstrap values (>90%) suggest strong support for that cluster. There is 100% support for the initial California and Florida cluster, for example, meaning this cluster appeared in 100% of the bootstrap trees. Note that the cluster

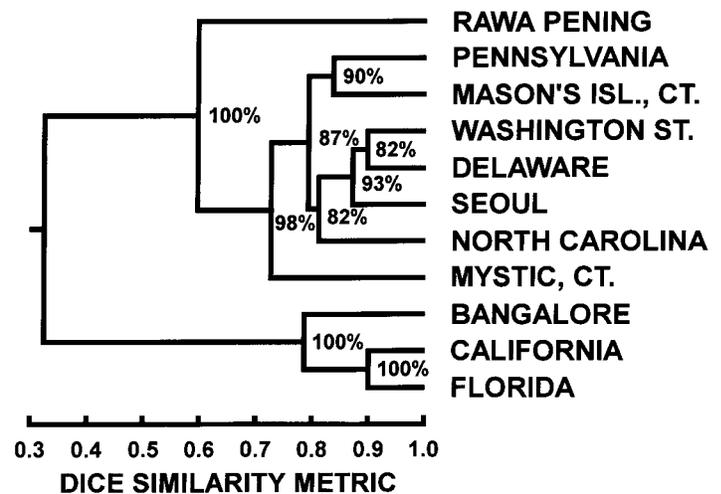


Figure 2. UPGMA (unweighted pair-group method, arithmetic average) phenogram generated from Dice Similarities for the five primer (111 marker) RAPD analysis. The percentages appearing to the right of each branch are the bootstrap for that cluster. High bootstrap values (>90%) suggest strong support for that cluster.

of the two monoecious controls, the Korean plant, and the four experimentals is supported by 98% of the bootstrap trees. However, the Mystic plant joined this cluster last, separating out in 87% of the bootstrap trees from the other U.S. monoecious. One might have expected this plant to group with its proximate geographic neighbor, the Mason Island plant. As we noted earlier, this plant, although washed well prior to DNA extraction, had a heavier concentration of epiphytic algae and bacteria, which may have produced artifactual bands, while necrosis may have degraded others, thus creating an outlier. Regardless, since the cluster including the Mystic collection separated out in 98% of the bootstrap consensus trees despite the presence of the Rawa Pening nearest neighbor, as identified by Madeira et al. (1997), there is strong evidence that the Mystic sample is of U.S. monoecious origin. It is important to note, however, that a separate introduction for the Mystic sample cannot be completely discounted as it is not possible to include all the potential diversity available internationally when assessing this outlier to the monoecious cluster.

Distribution. Known national distribution of hydrilla was mapped to 182 drainages in 16 states (Figure 3). Drainages designated as having hydrilla present indicate establishment in at least one site but do not imply occurrence throughout that hydrological unit. Available data comprised 2646 occurrence records for 685 sites. The majority of sites, 66%, were reservoirs, lakes or ponds, 30% were linear systems of rivers, streams, canals or ditches and the remaining 4% were marshes or other habitats. Pennsylvania is the latest state in which hydrilla has become naturalized. Since 1996 it has been found at the Schuylkill River, Philadelphia, [pers. obs.; Schuyler 8324 (PH)], at a reservoir in the Delaware River drainage [Schuyler 8345 (PH)] and at an impounded tributary in the upper Susquehanna River drainage [Colangelo 1998]. All three are distinct drainage basins. The most northern site reaches a latitude slightly higher (41°54'17"N) than where hydrilla was collected in Connecticut (41°21'40"N). Figure 3 may be used to identify areas that should be monitored for new infestations and areas where management should be implemented to prevent spread.

Although biotype has not been documented for each drainage, historically accepted and previously reported ranges of monoecious and dioecious hydrilla are presented in Figure 3. Within these main distributions, the regional biotype is assumed unless otherwise indicated.

Dioecious hydrilla dominates infested drainages of the southern Atlantic and Gulf Basins. Although present in small quantities at Lake Gaston, spanning the Virginia/North Carolina border, its northern Atlantic range generally stops at southern North Carolina. Infestations are extensive at lakes and waterways in both the Atlantic and Gulf Basins of Florida. Prominent Gulf Basin infestations include reservoirs in Alabama, marsh creeks of the Mobile Delta, lakes and marshes south of Louisiana Highway 1, and bayous and reservoirs westward to central Texas. Dioecious hydrilla occurs in the Interior Basin along the middle Tennessee River. In the western reaches of the Pacific Basin, dioecious hydrilla is scattered through California, and was eradicated from Arizona, where it occurred at two ponds in the mid 1980s (Everett Hall, Arizona Department of Agriculture, pers. comm.).

Monoecious hydrilla is distributed in drainages of the Atlantic Basin from Connecticut and Pennsylvania to central Georgia. The southern limit extends to Strom Thurmond Reservoir, on the Little River, and to a pond in the Upper Ocmulgee River drainage, Georgia. In the Interior Basin hydrilla is abundant at Deep Creek Reservoir, draining the Youghiogheny River of western Maryland and at several ponds near Asheville, North Carolina. Monoecious hydrilla is not known from drainages of the Gulf Basin. It appears sporadically in the Pacific Basin, occurring in California, most notably at Clear Lake (Anderson 1996) and in Washington, at the Lucerne/Pipe Lakes complex.

Dispersal of hydrilla. Mechanisms for tracking the occurrence and biotype of hydrilla have been presented herein. However, management requires that the introduction and regional dispersal of hydrilla be understood to prevent or slow further range expansion. The cost of range expansion can be substantial, especially in the semi-arid western states where water storage and delivery systems are critical. For example, the impact of hydrilla in the Imperial Valley, California, where irrigation supported nearly a billion dollar (gross revenues) economy, was enormous until grass carp was introduced (Stocker 1996).

Vectors for initial hydrilla introduction to a region are usually tied to the aquarium or water garden trade (Westbrooks 1990). In the case of dioecious hydrilla, it may have been first introduced to canals, ditches, ponds, lakes, and rivers by individuals and firms who wanted a year around, inexpensive supply of plants for the aquarium trade (McLane 1969). The aquarium trade functioned as a mechanism for the introduction of hydrilla to diverse regions, with populations identified during the 1970s in areas ranging from the Imperial Valley of California to the Louisiana lowlands. As late as 1984, Guerra (1984) noted a San Antonio lake where there was no boat traffic, but where both hydrilla and exotic aquarium fish were established. He stated that hydrilla could still be bought in aquarium shops. By 1990, Westbrooks (1990) reported that hydrilla was no longer being sold by aquatic plant dealers, at least not by any major firms. Therefore, the aquarium trade is probably no longer a major mechanism of dispersal for dioecious hydrilla.

Monoecious hydrilla was first reported in the Potomac river around 1980 (Haller 1982, Steward et al. 1984). Steward et al. (1984) reported it may have been introduced during transplanting and caging experiments in 1980 where hydrilla, misidentified as *Elodea canadensis*, was transplanted from Kenilworth Gardens. They further noted that Lilypons Water Gardens, a commercial supplier of aquatic ornamental plants in Maryland, may have been the source of the Kenilworth infestation. Haller (1982) reported that during a visit to Lilypons in 1980 he observed "a plant that had tubers and appeared very much like hydrilla". Steward and Van (1986) utilized monoecious plants collected from Lilypons for experimentation.

The monoecious biotype has a history of appearing at scattered, non-contiguous sites isolated from other populations. There is limited evidence that some of the monoecious hydrilla infestations may be the result of the importation via contaminated nursery stock of exotic water lilies. Ryan and Holmberg (1994) reported an infestation in a small aquatic

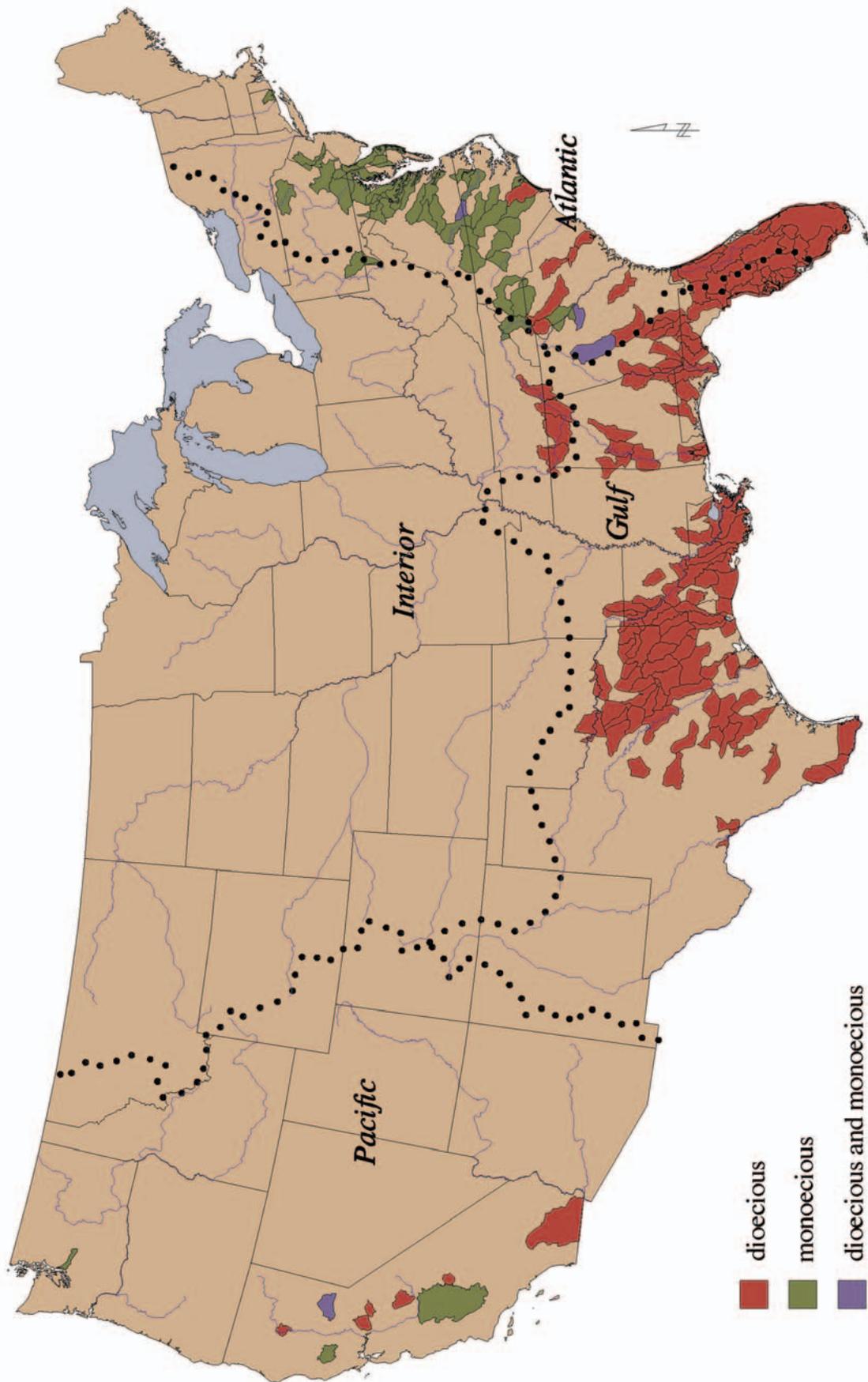


Figure 3. Distribution of hydrilla biotypes in U.S. drainage basins, January 2000.

nursery in Tulare County, California used for waterlily production. The California Integrated Pest Control Branch⁶ "... traced the source of the infestation to an aquatic nursery in Maryland. The nursery was cooperative in providing a list of customers that had been shipped plants in recent years. Subsequent checking of all of these customers by county agricultural biologists and Integrated Pest Control biologists found another small infestation in a small backyard pond in Santa Barbara County". The California Division of Plant Industry issued an advisor⁷ to the counties stating "It appears that the hydrilla was introduced in shipments of aquatic plants from Lilypons Water Gardens in Maryland." The advisor⁷ continued, "effective immediately, the Lilypons Water Garden facility in Maryland may only ship aquatic plant material into California when accompanied by an original state phytosanitary certificate issued by a Maryland Department of Agriculture official . . ." Robert Trumbule (Plant Protection and Weed Management Section, Maryland Department of Agriculture, pers. comm.), the scientist responsible for issuing Maryland's phytosanitary certificates, indicates that Lilypons has been diligent in trying to eliminate the contamination problem by cutting back the roots and washing the rhizomes of water lilies before shipping, as well as by draining and drying nursery ponds to reduce the hydrilla infestation.

As for the infestations studied with RAPD in this paper, Les et al. (1997) reported that white waterlily (*Nymphaea odorata* Ait.) is present at the Mystic infestation, while Kathy Hamel (Washington State Department of Ecology, pers. comm.) reports hybrid water lilies were present at Lake Lucerne. These reports certainly do not constitute any proof that water lilies were the vector for hydrilla introduction in these cases. Furthermore, reports of hydrilla infestations often occur years after the actual introduction so that even if they were imported on water lilies it is likely to have been before enhanced scrutiny of nursery materials began.

Additional human dispersal agents include the unintentional transport of small pieces of hydrilla on boat trailers, motor intakes, live wells, bait buckets, and draglines. This is probably the most common route of local dispersal. New infestations often occur adjacent to boat ramps, as observed when both biotypes first appeared at Lake Strom Thurmond, in 1995 (Michael Alexander, US Army Corps of Engineers, pers. comm.). Langeland and Sutton (1980) report that greater than 50% of fragments with 3 nodes can sprout while Steward (1992) reported 44% of dioecious and 20-44% of monoecious 3-node fragments exhibited regrowth.

Other dispersal mechanisms include the intentional distribution by individuals who, unaware of detrimental impacts, believe hydrilla will benefit sportfish or waterfowl habitat (Langeland 1996) and non-human dispersal agents. The non-human dispersal agents are difficult to document, but may include the transport of fragments, tubers, turions, and even seed by waterfowl (Joyce et al. 1980, Langeland 1996, Langeland and Smith 1984). Miller (1988) reports on a mo-

noecious infestation of an isolated pond in a waterfowl management area in Kent County, Delaware, where he believes waterfowl were the only possible vehicle for transmission.

We have presented tools that can be used to track and identify hydrilla infestations. Once the biotype has been identified the question may arise as to why this is important, that is, how do the biotypes differ biologically, and how do these differences impact management. Tubers of the U.S. monoecious biotype germinate at lower temperatures (Steward and Van 1987), after which growth spreads rapidly outward along the soil surface, producing a higher number of horizontal stems, root crowns, and higher shoot densities than dioecious U.S. hydrilla (Van 1989). It also produces tubers both more quickly than dioecious hydrilla and during long-day (summer) photoperiods (Van 1989). After a short-day (fall) induced burst of tuber production the monoecious hydrilla mat will often decline, then break loose from the substrate (Steward and Van 1987). This allows currents to transport the turion laden remaining fragments. This short-day decline is usually observed for monoecious cultures even in the subtropical climate at Ft. Lauderdale, Florida. These observations correlate with the annual growth habit of a temperate (colder weather) plant and with the report (Madeira et al. 1997) that the monoecious plant may have originated in Korea. The dioecious plant, on the other hand, produces larger tubers, in smaller numbers, and only under short day conditions. Upon germinating the growth is less lateral and more upwards towards the surface (Van 1989). This growth habit correlates with a more tropical origin, adaption to deeper waters and/or waters with less light penetration, as well as its probable origin in the Indian subcontinent (Madeira et al. 1997). While the dioecious plant is probably less likely to do well in northern climates (although it has overwintered in Iowa) the reverse is not necessarily true (McFarland and Barko 1999).

Once a new infestation of hydrilla is established, management is essentially the same. Herbicide applications of diquat, endothall (Steward and Van 1987), Aquathol-K, Hydrothal, and copper ethylenediamine (Steward and Van 1986) produced similar results for both biotypes. Since the monoecious biotype produces tubers even during long-day (summer) photoperiods herbicide treatment should begin as soon as possible after discovery (for new infestations) or several weeks after the tubers first sprout for known infestations. Intervention with the dioecious biotype should take place before short day tuber production begins (mid to late summer). Unfortunately new monoecious infestations are often not detected until later in the season when they reach the surface. Early intervention for new infestations is easier with dioecious hydrilla, as it is more likely to be noticed at the surface early, often before it commences tuber production. Repeated treatments are often needed for long term control, both within a growing season, and in consecutive growing seasons, because of newly sprouted tubers. Van and Steward (1990) have shown that monoecious tubers can remain dormant and viable for up to four years after formation. Haller et al. (1976) suggests that drawdowns, when possible, can be an effective tool for dioecious hydrilla control. He suggests timing the drawdown either to prevent tuber sprouting in the spring or to prevent tuber formation in

⁶1993 Annual Report of the Integrated Pest Control Branch, California Department of Food and Agriculture.

⁷Plant Exclusion Advisor: Hydrilla in Shipments of Aquatic Plant Material, Division of Plant Industry, California Department of Food and Agriculture, July 29, 1993.

the fall. Drawdowns against monoecious hydrilla are probably best conducted 2-3 weeks after tubers sprout since tuber production is not limited to the fall (Stratford Kay, North Carolina State University, pers. comm.). See Langeland (1996) for a more detailed review of management options.

The best approach for managers in regions without hydrilla may be that "an ounce of prevention is worth a pound of cure". As the example in this paper demonstrates, states may issue the requirement for a state phytosanitary certificate for each shipment of aquatic plants from facilities where contaminants have been demonstrated. This creates a monetary incentive for the facilities to "clean up". There is no doubt, however, that this procedure also places manpower and financial strain on the state certifying agencies. Therefore, it also reasonable that once a pattern of compliance is established, a return to self certification may be in order. At the Federal level, a 1999 amendment to the Federal Noxious Weeds Act (7 CFR Part 360.300) now clearly provides authority against interstate shipments of federally listed weeds (without a permit). Since hydrilla may spread as a contaminant on plants not requiring a permit, however, it is not clear to the authors how that authority can be exercised.

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ACKNOWLEDGEMENTS

The authors thank those who have reviewed this paper and offered their suggestions: Lars Anderson and F. Allen Dray, U.S. Department of Agriculture, Agricultural Research Service; Stratford Kay, North Carolina State University. The authors gratefully acknowledge those who have contributed occurrence information to the NAS database, with special thanks to: Les Ager, Georgia Department of Natural Resources; Charlie Biggar, Louisiana Department of Wildlife and Fisheries; Doug Blount, Mississippi Corps Project; Stratford Kay, North Carolina State University; Steve deKozlowski, South Carolina Department of Natural Resources; David DeMont, North Carolina Department of Environment, Health and Natural Resources; Eric Dibble, Mississippi State University; Mike Geihlsler, Georgia Department of Natural Resources; Rhandy Helton, Texas Parks and Wildlife Department; John Hunter, Charlotte County Environmental Services; John Kauffman, Virginia Game and Inland Fisheries; Robert Kipker, Florida Department of Environmental Protection; Cathy Martin, Delaware Division of Fish and Wildlife; Mike Naylor, Maryland Department of Natural Resources; Ross O'Connell, California Department of Food and Agriculture; John Odenkirk, Virginia Department of Game and Inland Fisheries, Doug Powell, Alabama Power Company; Ernesto Reyes, U.S. Fish and Wildlife Service; A. E. Schuyler, Academy of Natural Sciences; Ed Steinkoenig, Virginia Game and Inland Fisheries; John Tate, Virginia Department of Agricul-

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