

Root size and depth distributions for three species of submersed aquatic plants grown alone or in mixtures: evidence for nutrient competition.

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Introduction

Submersed plants play important roles in aquatic ecosystems (Carpenter and Lodge 1986). Introduced species may displace native species and alter aspects of ecosystem structure and function (Madsen 1997). Understanding competitive abilities of native and introduced aquatic plants may help in managing impacts of introduced species. Competitive interactions among submersed plants are poorly known (McCreary 1991, Gopal and Goel 1993). Results of competition experiments have been limited to a limited number of aquatic plant species (Hallier and Sutton 1975, Johnston et al. 1983, Tius and Stephens 1983, McCreary and Carpenter 1987, Moen and Cohen 1989, Spencer and Rejmanek 1989, Kautsky 1991, McFarland et al. 1992, Valley and Newman 1998, Van et al. 1998, 1999). In the case of submersed plants, there is conflicting evidence that either light (Madsen 1997, Madsen et al. 1991a,b) or nutrients (Chambers and Prepas 1990, Van et al. 1998, Van et al. 1999) may be important limiting factors in competitive interactions.

However, most studies have focused on changes in growth of aboveground parts, primarily shoots. This may be due to the difficulty of observing root growth in situ. Aquatic plant roots are involved in uptake of N, P, Fe, and micronutrients (Barbo et al. 1986, Barbo et al. 1991). Thus root growth and the associated ability to locate and exploit unevenly distributed patches of sediment nutrients have important consequences for a plant's competitive ability. McFarland et al. (1992) reported that increased density of either *P. nodosus* or *Hydrilla* (in monocultures or low soil fertility) was associated with an increased root to shoot ratio. Van et al. (1999) reported that *Hydrilla* and *Vallisneria spiralis* grown in mixtures allocated more biomass to roots when grown in low nutrient soils than when grown in high nutrient soils. Published results with terrestrial plants show that in some cases below ground competition may be equal to or greater than that associated with aboveground plant structures. Previous studies also suggest that plants alternately allocate resources to growth of roots or shoots, and that roots of different diameters may function differently in nutrient acquisition. For example Olson and Kemper (1993) suggested that small diameter roots have potentially greater uptake per unit surface area than larger roots. Boza and Oliver (1990) proposed that the fineness of *Xanthoxylum* roots was the basis for it being more competitive than *Glycyne max* for below ground resources. The purpose of this study was to evaluate changes in root growth over time for three species of submersed plants grown in monocultures or in mixtures. Specifically we sought to answer questions about the distribution of roots in the sediment, the relative abundance of roots of different size classes, and the relative timing of root and shoot growth. We also wanted to determine if the presence of competitors influenced these characteristics.

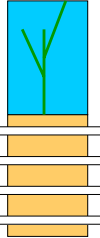


Figure 1. Plant Culture System with Minirhizotrons

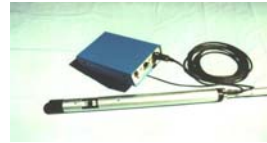


Figure 2. BTC 100X Minirhizotron Video Microscope

Species 1	Species 2 or Mixture	Probability for Fisher's Exact Test
<i>Hydrilla</i>	<i>P. pectinatus</i>	0.73
<i>Hydrilla</i>	<i>P. nodosus</i>	0.12
<i>P. nodosus</i>	<i>P. pectinatus</i>	0.02
<i>Hydrilla</i>	<i>P. pectinatus</i> + <i>Hydrilla</i>	0.05
<i>Hydrilla</i>	<i>P. nodosus</i> + <i>Hydrilla</i>	0.19
<i>P. pectinatus</i>	<i>P. pectinatus</i> + <i>Hydrilla</i>	<0.0001
<i>P. nodosus</i>	<i>P. nodosus</i> + <i>Hydrilla</i>	0.005
<i>P. pectinatus</i>	<i>P. pectinatus</i> + <i>P. nodosus</i>	0.0014
<i>P. nodosus</i>	<i>P. pectinatus</i> + <i>P. nodosus</i>	<0.0001

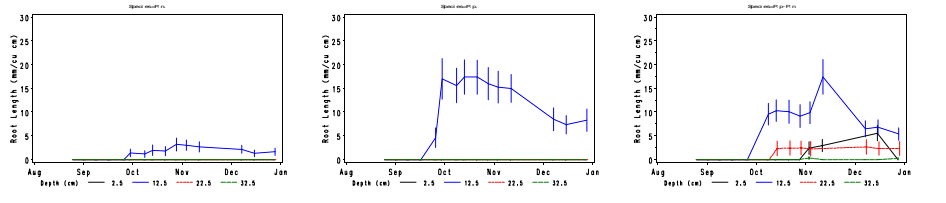


Figure 3. Root length density (RLD, mm³/cu cm) at four depths for *P. nodosus* (P.n.) and *P. pectinatus* (P.p.) alone or together (P.p. - P.n.).

Results and Discussion

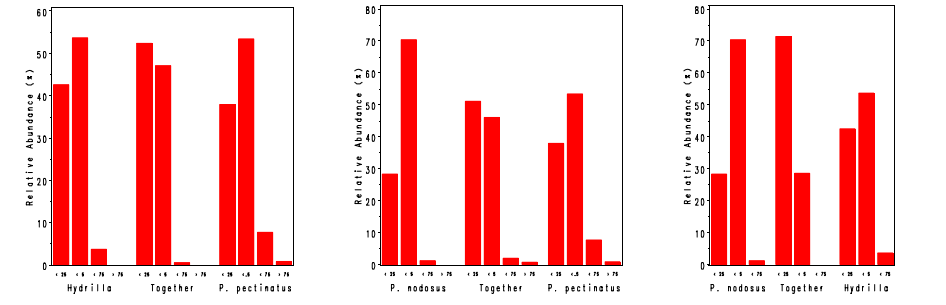
When grown as monocultures, plant dry weight varied from 43 to 57 g per container and was indicative of good growth (Table 1). In monocultures, each species produced vegetative propagules equivalent of from 6% to 23% of total dry weight and roots equivalent of from 3% to 12% of total dry weight. Plants grown in mixtures responded differently and indicated asymmetric effects of one species on another (Table 1). *Hydrilla* total dry weight decreased by 77% when grown in a mixture with *P. pectinatus*. Dry weight allocated to tubers and roots decreased. Dry weight of *P. pectinatus* grown with *Hydrilla* was essentially unchanged. *Hydrilla* grown with *P. nodosus* decreased by 85%. No vegetative propagules were produced by *Hydrilla* grown in a mixture with *P. nodosus*. When *P. nodosus* and *P. pectinatus* were grown together, *P. nodosus* dry weight decreased by 62% and *P. pectinatus* dry weight decreased by 15%. *P. nodosus* winter bud production increased slightly and *P. pectinatus* winter bud production decreased markedly.

Root distribution at different depths in the sediment was affected by the presence of plants of a different species (Figure 3). When grown in monocultures, *P. nodosus* roots were observed at 12.5 cm about 1 month after initiation of the experiment. Peak root length density (RLD) occurred in late October/early November and declined thereafter. A similar pattern was observed for *P. pectinatus*. *P. pectinatus* roots were only observed at 12.5 cm depth, but an important difference was that RLD for *P. pectinatus* was 8 to 10 times that of *P. nodosus*. RLD for *Hydrilla* was highest in early November and December and similar in magnitude to *P. nodosus*. Shallow roots (2.5 cm deep) were observed on two dates for *Hydrilla* grown in monoculture.

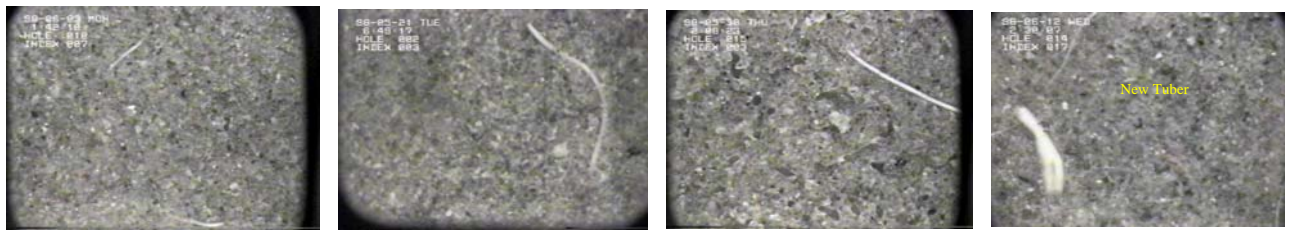
However when a given species was grown with plants of another species, roots were observed at depths other than 12.5 cm. When *P. nodosus* and *P. pectinatus* were grown together, RLD at 12.5 cm was less than the sum of that observed for monocultures. In addition, RLD at 2.5 cm and 22.5 cm increased beyond that at 2.5 cm. For mixtures of *P. pectinatus* and *Hydrilla* (data not shown), RLD at 12.5 cm was similar to RLD for *P. pectinatus* alone, but RLD increased at 22.5 and 32.5 cm. RLD was zero at these depths when either *P. pectinatus* or *Hydrilla* were grown in monocultures. In the case of *P. nodosus* and *Hydrilla* mixtures (data not shown), RLD was lower than for either species grown in monoculture and with the exception of one date was confined to a single depth of 12.5 cm. For two of three cases adding plants of another species changed the root depth distribution and increased the volume of soil occupied by roots. This finding is consistent with the interpretation that competitors of another species reduced the availability of sediment-based nutrients.

Figure 4 displays the distribution of root diameters across four size classes (< 0.25 mm; 2, >0.25 and < 0.5 mm; 3, >0.5 and < 0.75 mm; and 4, > 0.75 mm) for *P. pectinatus*, *P. nodosus*, and *Hydrilla*. The size distribution of *Hydrilla* roots did not differ from those of either *P. pectinatus* or *P. nodosus* (Table 2), but the frequency distributions for *P. pectinatus* and *P. nodosus* roots did differ (Table 2). *P. nodosus* had a greater proportion of roots in size class 2 and fewer in size class 1. The size distribution (based on diameter) of roots was also influenced by the presence of interspecific competitors. The size distributions of *Hydrilla* roots from monocultures were not significantly different from the distributions of mixtures that contained either *P. pectinatus* or *P. nodosus* plus *Hydrilla* (Table 2). However, size distributions of *P. pectinatus* roots from monocultures differed from those for mixtures that contained *P. pectinatus* and *Hydrilla*. There were more roots in the smaller size classes than would have been expected (Table 2). A similar result was observed for comparisons of *P. nodosus* monocultures with mixtures of *P. nodosus* and *Hydrilla*. Both *P. nodosus* and *P. pectinatus* in monoculture produced size distributions that differed from mixtures containing both species. Once again there appeared to be a shift in the abundance of smaller roots. Since smaller diameter roots are involved in nutrient uptake, a shift to smaller roots implies that the plants are adjusting to lower nutrient levels by producing more roots capable of this function. These results imply that competition for sediment-based nutrients may be occurring in these systems, and that in the case of mixtures of *P. nodosus* and *Hydrilla* and *P. pectinatus* and *Hydrilla* the competitive relationship may be asymmetric. Even though the present study dealt with monoculture *Hydrilla* and *P. pectinatus* as well as *P. nodosus*, this asymmetry is consistent with a previous report. McFarland et al. (1992), observed that dioecious *Hydrilla* grown in low nutrient sediment maximized shoot production at the expense of shoot elongation, while *P. nodosus* allocated proportionately greater biomass below ground to enhance nutrition.

Figure 4. Proportion of roots in four size classes for three aquatic plant species grown alone or together in combination. Size classes based on root width.



Images from the minirhizotron camera system.



Materials and Methods

These studies were conducted outdoors at Davis, California between August 25 and December 28, 1998. Three species of aquatic plants (*Potamogeton pectinatus*, *P. nodosus*, and *Hydrilla verticillata* (monococous type)) were grown in either monocultures or mixtures in PVC containers that were 1 m tall by 0.45 m diameter (Figure 1). The containers were filled to a depth of 58 cm with modified UC Mix (Spencer and Anderson 1986). Overlaying this was 42 cm of water. Four 5 cm diameter clear plastic tubes (minirhizotrons; see Taylor 1987) were mounted horizontally across the containers so that they were 2.5, 12.5, 22.5, or 32.5 cm below the surface of the sediment. The tubes were sealed in place to prevent water leakage. Ends of the tubes were closed with rubber stoppers and covered with aluminum foil to prevent light entry. Each container was planted with 8 sprouted propagules (four on each side of the plastic tubes). Three containers were monocultures and thus had 8 propagules of each of the above species. Containers with mixtures had four propagules of each type of plant in the mixture on each side of the minirhizotron tubes: *P. pectinatus* and *P. nodosus*, *P. pectinatus* and *H. verticillata*, and *P. nodosus* and *H. verticillata*. Propagules used in these studies were selected to be within the following fresh weight (fw) ranges: *P. pectinatus* tubers, 50 to 70 mg; *P. nodosus* winter buds, 250 to 300 mg; and *H. verticillata* tubers, 150 to 200 mg. Propagules were from cultures maintained at the EWRU facilities in Davis, and were allowed to sprout for one week prior to planting. At irregular intervals (approximately 1 to 2 weeks), we measured the height of the tallest plant in each container for each species, counted the number of flowers for each species, and the number of floating leaves for *P. nodosus* (minirhizotrons; see Taylor 1987) were mounted horizontally across the containers so that they were 2.5, 12.5, 22.5, or 32.5 cm below the surface of the sediment. The tubes were sealed in place to prevent water leakage. Ends of the tubes were closed with rubber stoppers and covered with aluminum foil to prevent light entry. Each container was planted with 8 sprouted propagules (four on each side of the plastic tubes). Three containers were monocultures and thus had 8 propagules of each of the above species. Containers with mixtures had four propagules of each type of plant in the mixture on each side of the minirhizotron tubes: *P. pectinatus* and *P. nodosus*, *P. pectinatus* and *H. verticillata*, and *P. nodosus* and *H. verticillata*. Propagules used in these studies were selected to be within the following fresh weight (fw) ranges: *P. pectinatus* tubers, 50 to 70 mg; *P. nodosus* winter buds, 250 to 300 mg; and *H. verticillata* tubers, 150 to 200 mg. Propagules were from cultures maintained at the EWRU facilities in Davis, and were allowed to sprout for one week prior to planting. At irregular intervals (approximately 1 to 2 weeks), we measured the height of the tallest plant in each container for each species, counted the number of flowers for each species, and the number of floating leaves for *P. nodosus*.

Concurrently with these measurements, we installed a specialized video camera (BTC-100X, Bartz Technology Co., Figure 2) into each of the minirhizotron tubes. The camera was connected to a video monitor and an 8 mm video recorder. The camera was used in conjunction with an indexing handle that moved the camera's position precisely one field of view with each increment. We examined 21 fields on the upper surface of each minirhizotron and recorded each field that had roots present. (For any given sampling date, we examined 4 tubes x 21 fields x 6 containers = 504 fields.) Videotaped images were later transferred to a computer via a video capture card. The digital images were analyzed manually using the image analysis program MOCHA v. 1.2 (Landel Scientific, San Diego). Using this software, we measured the following parameters for each root image: length, width, area, and average gray-scale intensity. Lengths were converted to root length density (RLD) by assuming that all roots within 2 mm of the surface of the tube were visible using the camera system. The volume of soil was calculated using this distance and the observation area (14 x 18 mm). RLD (mm³/cu cm) was calculated by dividing the total length of roots in a given image by the soil volume. Zero values were included in the final calculation of mean RLD at each depth for each date.

Abundance of different sized roots was determined by separating the roots into four size classes based on the measured diameters. Class 1 includes roots with diameters < 0.25 mm; class 2 had roots with diameters >0.25 and < 0.5 mm; roots with diameters > 0.5 and < 0.75 mm were assigned to class 3; and roots with diameters > 0.75 mm and < 1.25 mm were designated as class 4. To test the hypothesis that relative abundance of different size roots differed among species or with the presence of interspecific competitors, we used Fisher's exact test. To compare frequencies of roots for plants grown in mixtures with frequencies for plants grown in monocultures. All statistical analysis was with the SAS software package.

After 16 weeks, the experiment was terminated and the plants harvested. Dry weights (80°C, Coombs et al. 1985) of above ground parts were determined for each species. We used a plastic container (1057 cm³) to sample below ground parts to about 12.5 cm deep. One sample was collected on each side of the minirhizotron and the roots, rhizomes, and propagules separated, dried, and weighed as above. The number of roots of each species was also counted. We multiplied these values by the volume of sediment to a depth of 12.5 cm, divided by 1057 cm³ to obtain the weight or number of belowground parts per container.

Table 1. Plant dry weights and number of propagules for submersed plants grown alone or in mixtures. Values are grams or number per container.

Species	Alone				Together					
	Total	Rhizomes	Propagules	N	Total	Rhizomes	Propagules	N		
<i>Hydrilla</i>	44.90	0	1.57	10.40	401	10.37	0.95	0.57	1.71	167
<i>P. pectinatus</i>	42.97	3.34	5.12	7.29	702	44.61	2.76	1.11	8.04	866
<i>P. nodosus</i>	57.30	22.1	5.32	3.18	10.0	42.93	7.49	1.20	11.3	150
<i>Hydrilla</i>	44.90	0	1.57	10.40	401	6.91	0.47	0.03	0	0
<i>P. nodosus</i>	57.30	22.1	5.32	3.18	10.0	10.06	1.79	0.69	2.74	167
<i>P. pectinatus</i>	42.97	3.34	5.12	7.29	702	37.40	3.06	0.23	10.0	117

* For *Hydrilla* this value is for vegetative shoots.
 * For *Hydrilla* and *P. pectinatus* these are tuber; for *P. nodosus* these are winter buds.

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