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## Influence of pH and Medium Composition on Rooting of Hardy Deciduous Azalea Microcuttings

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*Additional index words.* *Rhododendron* sp., micropropagation, tissue culture

**Abstract.** Sphagnum peat (peat) media (adjusted to pH 4.0, 4.6, 5.5, 6.6, and 7.4 with ground dolomitic limestone) and unadulterated peat (pH 3.6) were tested for their effectiveness on rooting of hardy deciduous azalea (*Rhododendron* sp.) microcuttings in high-humidity chambers. Rooting of more than 90% occurred in media with pH 4.0, 4.6, and 5.5; however, a) shoot height and quality rating and b) root length and quality rating were superior at pH 4.0. Clonal differences in rooting percentages were found for 3 clones of azalea microcuttings rooted in 5 soilless mixtures. A mixture including equal parts (v/v) of peat and either sphagnum, vermiculite, or perlite, or a combination of 2 peat : 1 vermiculite : 1 perlite (by volume) increased rooting percentages over peat alone for all 3 azalea clones examined.

Adventitious root initiation in cuttings is controlled by a number of interacting factors (8). Nutritional factors (macroelements, microelements, carbohydrates, water), environmental factors (light, temperature, oxygen), plant factors (tissue age, physiological age, juvenility, degree of differentiation, disease status), and endogenous and exogenous substances (with auxins predominant) all participate in the complex mechanism of rhizogenesis (6, 7, 8, 11, 14, 18). If any one factor is limiting, the whole root formation process may be inhibited (8, 11, 18).

It is known from previous work (9, 10, 17) that rooting of cuttings is affected by the pH of the rooting medium. Paul and Smith (13) reported that peat containing from 37.5% to 75% of the saturated level of exchangeable Ca (corresponding to a pH of 4.4 and 6.6, respectively) enhanced rooting of chrysanthemum cuttings. In another report, Paul and Leiser (12) found that the highest rooting percentages (60-70%) of *Rhododendron* 'Red Wing' cuttings occurred when the peat contained from 2.7% to 54.2% of the saturated level of exchangeable Ca (corresponding to pH 4.38 and 5.60, respectively). Rooting of *Thuja occidentalis* cuttings was also affected by the medium's pH; the

best rooting occurred from pH 7.1 to 9.3 (2). The change in pH (from 6.0 to 11.0), due to calcium addition to the rooting solution, determined the type of callus which was formed and subsequently the rootability of *Populus balsamifera* cuttings (3). The diversity of rooting media, in addition to the physical properties important for rooting of cuttings, also provides a specific pH reaction, which meets the requirements of each plant species for improved rooting. Since microcuttings produced in tissue culture are considered miniature cuttings, the same factors influencing adventitious root initiation in cuttings could also affect microcutting rootability.

The objective of this work was to determine the influence of pH levels of a peat medium, modified with Ca additions, on rooting of azalea microcuttings. An additional objective was to find an appropriate soilless mixture which would meet the pH requirements for rooting of azalea microcuttings.

### Materials and Methods

**pH of the rooting medium.** Microcuttings were harvested from the 3rd reculture of shoot tip explant cultures of azalea accession 800374 (*Rhododendron* sp.). The culture conditions for shoot proliferation from explant establishment to 3rd reculture were those described elsewhere (4, 5). These microcuttings, 1.0-2.0 cm long, were inserted without any rooting hormone treatment directly into different pH peat media. The pH of the sphagnum peat was 3.6, and adjustments were made by incorporating different amounts of ground dolomitic limestone (CaCO<sub>3</sub> 45%, MgCO<sub>3</sub> 36%, "lime") into the peat moistened with distilled water, followed by thorough mixing.

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All media were kept in a high-humidity chamber for one week, then corrected further if needed to achieve desirable pH values. One week later, 5 rooting media were chosen with pH values of 4.0, 4.6, 5.5, 6.6, and 7.4, in addition to the untreated peat with a pH value of 3.6. Determinations of pH were made with a Corning 125 pH Meter on 5 cm<sup>3</sup> medium samples diluted with 10 cm<sup>3</sup> distilled water and mixed thoroughly for 15 min. Six 12 × 19 × 6 cm plastic packs were filled, each with one kind of peat medium, and 40 microcuttings per pack were stuck. The packs with the microcuttings were placed in a rooting chamber at 90–95% relative humidity, provided by fogging with distilled water for 30 sec every 30 min.

The rooting temperature was adjusted to 26° ± 1°C by thermostatically controlled bottom heat from electric cables. Illumination of 85 μmol·s<sup>-1</sup>·m<sup>-2</sup> (400–700 nm) for 24 hr was provided with cool-white fluorescent lamps. The microcuttings were kept for 3 weeks under these conditions for root initiation and then moved to another chamber for root development. The 2nd rooting chamber had the same environmental conditions as the first, except the relative humidity was adjusted to 70–80%. After 3 weeks in this environment, the rooting of microcuttings was evaluated.

Data taken included the number of microcuttings rooted, length of the roots, shoot growth of the rooted microcuttings, and quality ratings of root development and of shoot growth. Since all 40 microcuttings per treatment did not root, only data of 20 random rooted microcuttings were taken for statistical analysis. Shoot and root ratings were evaluated on a scale of 1.0 to 3.0, with 1.0 referring to limited shoot growth or poor root system development and 3.0 referring to vigorous shoot growth and heavy and uniform development of roots around the microcutting base.

**Rooting medium composition.** Five different soilless media were tested (all on a volume basis) — 1 peat : 1 sphagnum; 1 peat : 1 vermiculite (#2); 1 peat : 1 perlite; 2 peat : 1 vermiculite : 1 perlite; and peat alone. Rooting media were drenched with distilled water and placed in plastic packs of 12 × 19 × 6 cm inside dimensions. Forty 1.0–2.0 cm microcuttings were harvested from the 2nd reculture of shoot-tip explants of the clones accessions 800374, 620014, and 800057 (*Rhododendron* spp.) and were inserted in packs containing each rooting medium. The packs with the microcuttings were placed for 3 weeks in each one of the 2 rooting chambers with the environmental conditions mentioned previously. After the 6-week rooting period, the experiment was terminated, and rooted microcuttings were counted. The pH of each medium was measured before microcutting insertion and also after microcutting lifting.

## Results

**Effect of pH on microcutting rooting.** The pH of the medium had a marked effect on rooting percentage of accession 800374 microcuttings (Fig. 1). The poorest percentage of rooting (55.0%) occurred in peat alone (pH 3.6). Rooting was improved in the pH range from 4.0 to 5.5 (97.5% and 85.0%) by incorporating lime into the medium. At pH 6.6 the rooting percentage decreased but still was higher than that of peat alone (70.0% vs. 55.5%). The highest rooting percentages (97.5%) were achieved at pH 4.0 and 4.6.

**Effect of pH on shoot and root length.** The curve of root length paralleled that of shoot length for the 6 pH levels; moreover, each curve exhibited a peak at pH 4.0 (Fig. 2, top). Media with pH 5.5, 6.6, and 7.4 entirely inhibited microcutting growth.

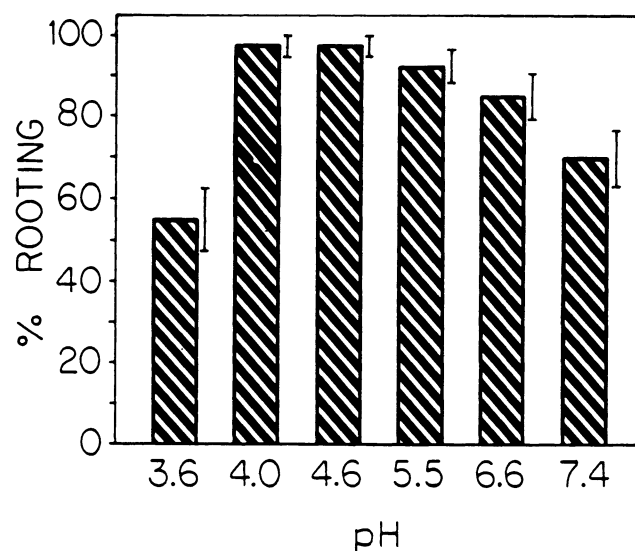


Fig. 1. Effect of the medium pH on rooting of azalea accession 800374 microcuttings. Forty microcuttings per treatment. Bars indicate SD.

In addition only a few short roots developed at the microcutting bases for these pH levels.

**Effect of pH on shoot and root quality rating.** The same pattern was obtained for shoot and root quality rating as that for shoot and root length. Furthermore, the peaks for each curve occurred at the same pH (4.0). The poorest shoot and root quality ratings were obtained at pH 6.6 and 7.4 (Fig. 2, bottom).

**Composition of rooting medium.** The pH of all 5 media measured 6 weeks after microcutting insertion exhibited small increases compared to the pH at the time of microcutting insertion (Table 1). Peat alone and peat plus sphagnum had the lowest beginning and ending pH, whereas higher pH levels were achieved in media containing peat in combination with perlite, vermiculite, or both.

Differences were found in percentages of microcutting rooting among the 3 clones for each rooting medium tested. In general, peat showed the lowest rooting percentage (mean of all 3 clones; 54.2%), whereas the medium of 2 peat : 1 vermiculite : 1 perlite had the highest (mean of all 3 clones; 78.3%). The 3 other media scored intermediate percentages of microcutting rooting (Table 1).

## Discussion

Root formation in cuttings, which involves a sequence of morphogenetic phenomena, could be grouped for practical reasons in 2 stages: 1) root primordia initiation and development and 2) root emergence. Each stage has its own requirements for nutrients and growth substances (6, 11).

The principal exchangeable ion held by the peat is hydrogen, present mainly as COOH (12, 13). Addition of Ca to peat causes a decrease in exchangeable and soluble hydrogen and an increase in exchangeable and soluble Ca (12, 13). Thus, the pH of the peat became less acidic, and Ca ions were supplied, which are necessary for functions of the developing cells. One interpretation for the differences found in rooting of azalea microcuttings at varying levels of pH may be related to an alteration of endogenous 1H-indole-3-acetic acid (IAA) action or IAA influence on mitotic activity during root primordia development. Moreover, metabolic events during root primordium initiation could be affected by pH changes (15, 16).

Table 1. Changes in pH and rooting of microcuttings of azalea accessions 800374, 620014, and 800057 in different media.

Rooting medium (proportions v/v)	pH <sup>z</sup>		Rooting <sup>y</sup> (%)			
	Beginning	Ending	800374	620014	800057	Mean
Sphagnum peat (peat)	3.6	3.7	52.5 ± 7.9*	50.0 ± 7.9	60.0 ± 7.7	54.2 ± 4.5
Peat + sphagnum (1:1)	3.7	3.9	65.0 ± 7.5	80.0 ± 6.3	65.0 ± 7.5	70.0 ± 4.2
Peat + vermiculite (1:1)	3.8	4.1	75.0 ± 6.8	82.5 ± 6.0	57.5 ± 7.8	71.7 ± 4.1
Peat + perlite (1:1)	3.9	4.0	70.0 ± 7.2	72.5 ± 7.1	72.5 ± 7.1	71.7 ± 4.1
Peat + vermiculite + perlite (2:1:1)	3.8	4.1	72.5 ± 7.1	82.5 ± 6.0	80.0 ± 6.3	78.3 ± 3.8

<sup>z</sup>Data taken prior to sticking and after 6 weeks.

<sup>y</sup>Six weeks in rooting medium.

\*Mean of 40 microcuttings per treatment ± SD.

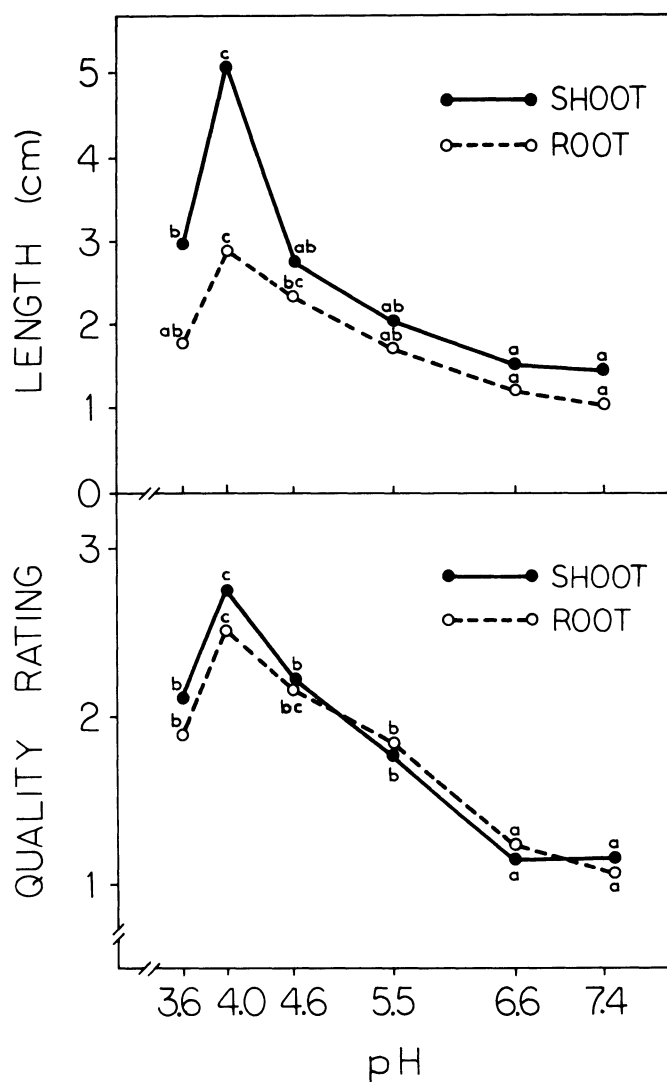


Fig. 2. Shoot and root growth of azalea accession 800374 microcuttings rooted at different pH levels. Each point represents the mean of 20 rooted microcuttings. Mean separation within curves by HSD, 1% level.

Rooting of azalea microcuttings occurred in a range from pH 4.0 to 6.6, although the best shoot growth and root development were restricted to pH 4.0. Usually, successful rooting of azalea microcuttings is associated with a flush of new growth. Pierik and Steegmans (14) reported that pH of the medium from 4.0 to 6.0 did not influence rooting of *Rhododendron* stem explants.

Different plant material and technique could account for this disagreement, since inverted leafless stem segments were used. At pH 6.6 and 7.4, root expansion of azalea microcuttings was limited and shoot growth was inhibited. This agrees with research of Paul and Leiser (12) who reported root length of 'Red Wing' *Rhododendron* cuttings was also depressed with addition of 71.4% and 88.5% exchangeable Ca in the peat (pH 5.9 and 7.0). The percentage of rooting and the index of root quality of *Thuja occidentalis* cuttings increased from pH 5.1 to 7.1 and reached a plateau from 7.1 to 9.3 (2). No rooting at an extremely high pH was reported by Cormack in balsam poplar cuttings (3). He suggested that adventitious roots did not emerge at pH 11.0, even though root primordia had been developed, because of the surrounding dense and hard callus due to calcified middle lamella from the excess Ca in the medium. In some unrooted azalea microcuttings, callus aggregation at the higher pH level was observed.

Rooting was reduced in peat (pH 3.6–3.7) and increased in peat plus vermiculite or perlite or both (pH around 4.0). The different rooting percentages obtained in the different soilless mixtures are probably related to the differences in pH, nutritional factors, or physical properties (porosity and water-holding capacity), or a combination. Varying rooting percentages in different rooting mixtures were also reported by Anderson (1) for *Rhododendron* microcuttings.

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## Soil Cultivation and Incorporation Effects on the Edaphic Properties of Turfgrass Thatch

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*Additional index words.* Kentucky bluegrass, creeping bentgrass, cation exchange capacity

**Abstract.** Cultivation practices have been used to control thatch, principally by removal of organic constituents and by incorporation of soil to promote decomposition of the organic residues. The purpose of this study was to determine the influence of soil incorporation on the edaphic properties of turfgrass thatch. Field studies were performed on 2 creeping bentgrass (*Agrostis palustris* Huds.) and 2 Kentucky bluegrass (*Poa pratensis* L.) sites. Treatments included core cultivation and vertical mowing, separately and in combination; after 6 weeks, samples of the thatch and thatch-like derivatives were taken for determining ash content, bulk density (BD), cation-exchange capacity (CEC), and pH. Soil incorporation into thatch reduced the percentage of ash content and CEC, but increased BD and CEC·BD — an expression of CEC on an undisturbed volume basis. The extent to which these changes occurred reflected the amount of soil incorporated by the number and type of cultivation methods employed.

Thatch has been regarded as a largely negative feature in turfgrass systems. Undesirable effects associated with thatch include increased susceptibility to cold, heat, and drought injury and increased severity of disease and insect problems. Traditionally, emphasis has been placed on methods to control or remove thatch from lawns and other turfs. In situations of excessive thatch accumulation, however, the complete removal of the thatch may result in severe injury to the turfgrass community. This damage is due to a large percentage of the turfgrass root systems that may be within the thatch layer (4).

One method for thatch modification or reduction is soil incorporation. Research has shown the most effective method for reducing the thatch layer is soil topdressing (3, 7). Addition of soil presumably improves the environment for microbial activity. Topdressing, however, is uneconomical for large turf areas (5).

Core cultivation is a more feasible method of soil incorporation on large turfgrass areas. This method is a gradual process for reducing thatch. Murray and Juska (5), in a 9-year study,

found coring had no effect on thatch decomposition in the first 5 years. Smith (6) found in a short-term study that coring did not adequately control thatch. Murray and Juska (5), however, reported that 5 years of coring significantly reduced the thatch layer. What short-term effect soil incorporation had on the physical and chemical properties of thatch was not determined.

In laboratory studies, Hurto et al. (4) found thatch to have 1) low-moisture retention due to the predominance of macropores and 2) low-bulk density. Hurto et al. (4) found the addition of soil to thatch increased moisture retention and bulk density. Danneberger et al. (2) found thatch to have a low CEC. The effect of soil incorporation on increasing the CEC of thatch has been hypothesized (1). The purpose of this research was to assess quantitatively the effects of soil incorporation on thatch in the field.

### Materials and Methods

Four field studies were initiated in Sept. 1978 and Apr. 1979 at 3 locations in Illinois; 2 studies were conducted in Urbana, one in Champaign, and one in Oak Brook.

Urbana studies were conducted on a 5-year-old 'Penncross' creeping bentgrass turf with a  $\pm 2$ -cm thatch layer. The underlying soil was a Flanagan silt loam (Aquic Argiudoll). The grass was mowed at 0.6 cm height 4 times per week during the growing season. Nitrogen was applied in April, June, August, and September using a 10N–2.6P–3.2K water-soluble fertilizer in sufficient quantity to supply a total of 200 kg N/ha/year. Levels of P and K were adequate according to soil-test results obtained from the Univ. of Illinois Soil Testing Laboratory. The soil-test

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