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Screening Commercial Peat and Peat-based Products for the Presence of Ericoid Mycorrhizae[1]

Nicole R. Gorman[2] and Mark C. Starrett[3]
Department of Plant and Soil Science
University of Vermont, Burlington, VT 05405

Abstract

Pieris floribunda (Pursh) Benth. & Hook., a known host of ericoid fungi, was used as a model plant to investigate the presence of ericoid mycorrhizal fungi in select peat and peat-based products. After growing in each medium for 75 days, roots of seedlings were examined and average percent colonization was determined for each sample. Results indicate that these fungi are present in the majority of peat and peat-based media tested. Seedlings grown in some of the selected media had a greater percentage of root cells colonized by ericoid mycorrhizae than others in the study.

Index words: Ericaceae, mountain andromeda, *Pieris floribunda*.

Species used in this study: mountain andromeda, [*Pieris floribunda* (Pursh) Benth. & Hook.].

Significance to the Nursery Industry

The nursery industry uses sphagnum peat (peat) as a major component in media used in the production of ericaceous plants. In nature, roots of plants in the heath family (Ericaceae Juss.) form symbiotic associations with fungi called, 'ericoid mycorrhizae'. These mycorrhizae assist the host plant mostly in mineral nutrient acquisition. For this reason, growers of ericaceous plants should consider selecting a growing medium which naturally contains ericoid mycorrhizae. In this study, the majority of peat and peat-based media investigated contained ericoid mycorrhizal fungi. However, the extent of roots colonized by the ericoid mycorrhizae differed for each medium examined. Further studies must be conducted to determine whether increased colonization is correlated with peat source and whether increased colonization affects the growth of the host plant during production and following installation in the landscape. Additional studies should also determine which species of ericoid mycorrhizae are present in each geographic source of commercially-harvested peat.

Introduction

*Pieris floribunda (*mountain andromeda) belongs to the family Ericaceae. This species, along with other members of the family, such as *Calluna* Salisb. (heather), *Kalmia* L. (laurels), *Rhododendron* L. (rhododendron), and *Vaccinium* L. (blueberries and cranberries) (21) are important plants in the landscape and quite valuable in the nursery industry.

Ericaceous plants have a unique, structurally reduced root, lacking root hairs (26). It is hypothesized that this reduced root has co-evolved with species of fungi that together form the mutualistic symbiosis known as 'ericoid mycorrhizae' (12, 27). Experiments using ericoid fungi have demonstrated their ability to aid in nitrogen and phosphorous uptake by the host plant (2, 3, 13, 17, 28, 29, 30). Use of commercial growing media containing ericoid mycorrhizal fungi may reduce current fertilizer requirements of ericaceous species without decreasing plant productivity.

In the wild, members of the Ericaceae are often found growing in nutrient-poor areas with high carbon:nitrogen (C:N) ratios, acidic soil and seasonally high water tables (26). The native soil-type is characterized by high levels of organic matter which is often recalcitrant due to high phenolic acid and tannin concentrations, or anoxic conditions caused by a high water table and/or low ambient temperatures due to high altitude or latitude (11, 13, 22, 26). Ericoid fungi can release nutrients from organic matter that are generally unavailable to plants and allows the host to survive in otherwise harsh edaphic conditions (7, 8, 18, 23).

Due to heightened awareness of environmental conservation, the amount of fertilizer used in the commercial production of landscape plants is being monitored more closely. Exploitation of the benefits of ericoid mycorrhizae in their capacity to aid in nutrient uptake may allow for decreased fertilizer applications during production (31). Container nursery stock production of plants in the Ericaceae typically involves the use of a growing medium containing peat (15). An increase in growth and productivity was demonstrated when peat containing ericoid fungi was used as a propagation medium (9, 19, 20). Some evidence suggests that early contact between host and fungus serves to detoxify the rhizosphere and to help establish the young host plant (14). In fact, early association of the host with a fungal partner may be vital to host survival in the wild (1, 14).

The objective of this study was to determine whether ericoid fungi are present in select peat and peat-based commercial growing media. Plants were only evaluated for the presence of ericoid fungi in the growing media. No attempt was made to determine any growth response of the seedlings due to the complications associated with the use of both commercial growing mixes containing fertilizers and pure sphagnum peat which did not contain fertilizer or other medium amendments.

Materials and Methods

Seed preparation and germination. Seed of *Pieris floribunda* was collected from a native population of open-pollinated plants growing in Haywood County, NC. Seeds were

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[2]Former Graduate Assistant.

[3]Assistant Professor. To whom reprint requests should be addressed. E-mail address: <Mark.Starrett@uvm.edu>.

cleaned and counted under a dissecting microscope to select seeds of similar size, color and fullness.

On June 19, 1997, seeds were surface-disinfested with 30 ml (1 fl oz) of a dilute bleach solution containing 1.05% sodium hypochlorite (Clorox Bleach, The Clorox Co., Oakland, CA). Polyoxyethylene sorbitan monolaurate [(Tween 20) Fisher Scientific, Fair Lawn, NJ] was added (0.05%) to the sodium hypochlorite solution as a surfactant. During disinfestation, seed was stirred vigorously on a stir plate (Barnstead/Thermolyne, Dubuque, IA) for 15 min. Under aseptic conditions, surface-disinfested seed and the solution of sodium hypochlorite was decanted into a Buchner funnel lined with No.1, 70-mm (2.8 in) filter paper (Whatman International Limited, Kent, England) and rinsed with three serial washes [≈ 150 ml (5 fl oz) sterile distilled water each]. Bleach solution and wash water was vacuumed through the funnel using a Doerr pump (Fisher Scientific, Fair Lawn, NJ). Seeds were collected from the surface of the filter paper and transferred to 60 × 15-mm (2.4 × 0.6 in) polystyrene disposable petri dishes containing 10 ml (0.3 fl oz) sterile water agar [6 g/liter (0.7 oz/gal)] (JRH Biosciences, Lenexa, KS). The dishes were sealed with Parafilm 'M' (American National Can, Greenwich, CT) and enclosed in 950 ml (1 pt) nonvented, polyethylene storage bags. The bags were placed in a controlled-environment chamber (Percival, Inc., Boone, IA) maintained at days/nights of 22/20C (72/68F). A 16-hr photoperiod was provided by six, high-output cool-white fluorescent lamps [GE Model F24T12-CW-HO (GE Lighting, Cleveland, OH)] and two 60-W incandescent bulbs suspended 18 cm (7 in) above the dishes. Lamps and bulbs provided an average photosynthetic photon flux [PPF (400–700 nm)] of 251 □mol/sq m/sec as measured at the top of the dishes. These and all other light measurements were taken with a LI-COR LI-190 Quantum Sensor and recorded with a LI-COR 1000 Data Logger (LI-COR, Lincoln, NE). Petri dishes were scanned weekly for contamination. Contaminated dishes were removed from the bags and discarded.

Growing media preparation. Select commercial growing media were obtained from suppliers in the northern United States and Canada. Media were chosen based on both geographic location of peat harvest and volume of product sold to commercial growers. The media utilized included: Fafard 3B (Conrad Fafard, Inc., Agawam, MA), Garden Magic Michigan Peat (Michigan Peat Company, Sandusky, MI), Garden Magic Peat Baccto (Michigan Peat Company, Sandusky, MI), Garden Magic Sphagnum Peat Moss (Michigan Peat Company, Sandusky, MI), Lerner Peat Moss (Maximillian Lerner Corp., NY), Northern Bay Peat '2' [harvested from a depth of ≈2 ft (0.6 m) (Northern Bay Peat LLC, Penobscot, ME)], Northern Bay Peat '7' [harvested from a depth of ≈7 ft (2.1 m) (Northern Bay Peat LLC, Penobscot, ME)], Northern Bay Peat sphagnum [harvested from the bog surface (Northern Bay Peat LLC, Penobscot, ME)], Premier ProMix (Premier Horticulture, Quebec, Canada), Premier Tourbe Pro-Moss horticulture (Premier Horticulture, Quebec, Canada), Premier Tourbe TBK from Baie Comeau (Premier Horticulture, Quebec, Canada), Premier Tourbe TBK from Rogersville (Premier Horticulture, Quebec, Canada) and Scotts Metro Mix 360 (The Scotts Company, Marysville, OH) (Table 1).

Synthesis of mycorrhizae. On July 16, 1997, one-hundred-four GA7 Magenta boxes (Magenta Corp., Chicago, IL) were each filled with approximately 200 ml (6.8 fl oz) of one of the aforementioned thirteen selected commercial growing media. Each medium was moistened with varying amounts of sterile, distilled water in order to achieve a uniform moisture content of approximately 70% of capacity (Table 1). Uniform seedlings were transplanted into Magenta GA7 boxes, with 1 seedling per container. To provide adequate mineral nutrients to all media to ensure initial growth and establishment of the seedlings, 3 ml (0.1 fl oz) of sterile, liquid Woody Plant Medium [WPM (16)] supplemented with 200 mg/liter (0.023 oz/gal) NaH_2PO_4 and 80 mg/liter (0.01 oz/gal) adenine hemisulfate was added to each box. The WPM was adjusted to pH of 5.2 with 1N HCl prior to addition to boxes. Boxes were covered with Magenta GA7 vessel covers and sealed with Parafilm 'M'. Sealed boxes were placed in a Sherer (Rock Hill, SC) modified refrigeration unit maintained at days/nights of 25/20C (77/68F). A 16-hr photoperiod was supplied by twelve, Power Groove cool-white fluo-

Table 1. Commercial peat and peat-based growing media investigated for the presence of ericoid mycorrhizal fungi, their geographic source and amount of water added to hydrate each medium to a moisture content 70% of capacity.

Growing medium	Location of harvest	ml of water[z] (fl oz)
Fafard 3B	New Brunswick, Canada	6.6 (0.22)
Garden Magic Michigan Peat	Michigan, USA	13.5 (0.46)
Garden Magic Peat Baccto	Michigan, USA	2.4 (0.08)
Garden Magic Sphagnum Peat Moss	Michigan, USA	1.6 (0.05)
Lerner Peat Moss	Quebec, Canada	9.9 (0.33)
Northern Bay Peat 2[y]	Maine, USA	0.0 (0.00)
Northern Bay Peat 7[x]	Maine, USA	53.1 (1.80)
Northern Bay Peat sphagnum[w]	Maine, USA	6.1 (0.21)
Premier ProMix	New Brunswick and Quebec, Canada[v]	12.5 (0.42)
Premier Tourbe Pro-Moss Horticulture	Quebec, Canada	8.5 (0.29)
Premier Tourbe TBK Baie Comeau	Quebec, Canada	4.6 (0.16)
Premier Tourbe TBK Rogersville	New Brunswick, Canada	3.8 (0.13)
Scotts Metro Mix 360	New Brunswick, Canada	6.3 (0.21)

[z]Water added per 0.007 cu ft (0.0002 cu m) of growing medium to achieve ≈ a moisture content 70% of capacity.

[y]Peat harvested from a depth of approximately 2 ft (0.6 m).

[x]Peat harvested from a depth of approximately 7 ft (2.1 m).

[w]Peat harvested from the bog surface.

[v]Peat harvested from select locations in New Brunswick and Quebec and blended during production of growing medium.

Table 2. Mean percent root colonization of *Pieris floribunda* by ericoid mycorrhizal fungi present in select peat and peat-based commercial growing media

Growing medium	Mean[z]	SD
Northern Bay Peat 2.25 m[y]	56.2a[x]	19
Premier Tourbe Pro-Moss Horticulture	50.3ab	26
Premier Tourbe TBK Baie Comeau	48.0ab	20
Garden Magic Sphagnum Peat Moss	45.7abc	25
Northern Bay Peat 7 m[w]	44.3abc	28
Lerner Peat Moss	38.2abc	20
Premier ProMix	35.2abc	33
Northern Bay Peat sphagnum[v]	33.0abc	18
Premier Tourbe TBK Rogersville	29.8abc	20
Garden Magic Michigan Peat	22.4cd	26
Scotts Metro Mix 360	8.1de	13
Garden Magic Peat Baccto	0.5e	2
Fafard 3B[u]	—	—

[z]Means are based on eight replicates. In the event all replicates failed to survive, means were calculated from living plants only. Percent colonization based on three random views per seedling.

[y]Peat harvested from a depth of approximately 2 ft (0.6 m).

[x]Means not sharing a common letter are significantly different (Fisher's LSD, $p < 0.05$).

[w]Peat harvested from a depth of approximately 7 ft (2.1 m).

[v]Peat harvested from the bog surface.

[u]Statistical analysis not performed due to 100% mortality of the seedlings.

rescent lamps (GE Model F96PG17-CW) and four, 60-W incandescent light bulbs suspended 50 cm (20 in) above the tops of the boxes. Lamps and bulbs provided an average PPF of 268 □mol/sq m/sec as measured at the top of the boxes.

Harvest. On September 28, 1998, 75 days after placement of seedlings into the controlled environment chamber, the study was terminated and roots harvested. Root samples were prepared to determine fungal colonization. Each root sample was placed in a beaker and gently washed in distilled water. The samples were then cleared and stained following a protocol adapted from Brundrett et al. (6) and examined using bright field microscopy. To quantify root colonization, the entire root system for each plant was spread evenly over the surface of a glass microscope slide and the roots then covered by a solution containing lactic acid, glycerol and water (1:1:1 by vol). The roots and solution were then covered with a 22 × 40 mm (0.9 × 1.6 in) glass cover slip. Individual roots were randomly selected and examined for the presence of infected cells. Based on a preliminary study, three random views at ×400 provided a consistent means of assessing mycorrhizal colonization of roots. Colonized cells were determined as a percentage of all cells within each view. If a random view did not contain a root, another random view was selected. Average percent colonization was determined as the mean of the three counts per plant.

Experimental design and statistical analysis. The experimental design was a randomized complete block (RCBD) which consisted of eight replicates (blocks), that were conducted simultaneously. Each replicate contained thirteen boxes. Each box represented one peat product of each of the thirteen commercial media tested. The thirteen boxes were randomized spatially within each replicate. Each box contained one seedling. For each seedling, an estimate of intensity of colonization was based on the average of three randomly determined ×400 views of selected hair roots, each resulting in an estimate of percent of root cells colonized. Comparisons of colonization intensity (i.e., mean percent colonization) are based on analysis of variance (ANOVA). In order to satisfy the homogeneity of variance assumption associated with the analysis of variance, data corresponding to percent colonization was arcsine square-root transformed. Although statistical significance was based on transformed data, means presented in the table represent non-transformed data (Table 2). Statistical analyses were performed using GLM and LSMEANS in SAS (24, 25). Pairwise comparisons were based on Fisher's LSD ($p < 0.05$).

Results and Discussion

Ericoid fungi colonized the roots of seedlings grown in all of the peat products evaluated (Table 2). The seedlings grown in Fafard 3B were not included in the statistical analysis as none of these seedlings survived to harvest.

Significant differences were detected in mean percent colonization between peat products. Northern Bay Peat 2.25m, Premier Pro-Moss Horticulture, and Premier TBK Baie Comeau were colonized more intensely than Garden Magic Michigan Peat, Scotts Metro Mix and Garden Magic Peat Baccto.

Seedlings grown in Garden Magic Peat Baccto were among the least colonized when compared to those growing in the other commercial growing media. Surprisingly, this product is the only medium tested that is labeled as containing beneficial live microorganisms. This result demonstrates the importance of determining what microorganisms the medium contains and how each might benefit the crop during production.

There was no evidence to indicate whether depth of harvest affects fungal population and colonization. Only a single product, 'Northern Bay Peat', harvested from three soil depths was tested. The results show that the seedlings grown in the peat harvested from a depth of 2 ft (0.6 m) or 7 ft (2.1 m) were similar to those seedlings growing in peat harvested from the soil surface, 'Northern Bay Peat Sphagnum' (Table 2). The lack of an effect of harvest depth on intensity of root colonization is somewhat surprising considering the majority of roots on ericaceous plants grow near the surface of the soil and are typically mycorrhizal (26). One would expect that ericoid roots harvested along with the peat would provide an inoculum source that would otherwise not be found in peat harvested from lower strata. The authors have used roots colonized with the ericoid mycorrhizal fungus, *Hymenoscyphus ericae*, as a source for rapid production of inoculum (data not presented).

Many variables can affect the population of ericoid fungi in growing media. First, peat is often harvested from regions where ericaceous plants are found naturally (14). Peat from different geographical regions may contain different species or strains of ericoid fungi, some perhaps are more infective than others. Goulart et al. (10) surveyed native and commercial *Vaccinium* populations in the northeastern and midwestern United States and found a high degree of variation in infection. Additionally, peat for horticultural purposes is extracted from various soil depths ranging from the surface layer to depths of more than 7 ft (2.1 m). The majority of ericaceous roots exist at or near the surface, thus ericoid fungi may be more likely to be present at shallow depths (26). However, ericoid fungi have been shown to possess

considerable saprophytic capabilities, and therefore may be able to survive at greater depths without the presence of a host (22). Also, there are different methods for processing peat and manufacturing growing media. Some use heat- or steam-pasturization to rid the components of harmful microorganisms. Unfortunately, this practice can destroy beneficial fungi, bacteria and other microbes (15). Finally, the season of peat harvest may affect fungal populations. Several studies have revealed that colonization intensity can fluctuate throughout the year, increasing with new root formation in late summer and fall and decreasing during winter with a gradual increase through summer (5). Berta and Bonfante-Fasolo (4) noted that deciduous species have varying levels of infection. Peat may be harvested at times when fungal populations are low and consequently may pass this characteristic on to the growing medium in which it is incorporated.

While this study shows that commercially-available peat and peat-based products contain ericoid mycorrhizal fungi, further research must be conducted to determine exactly which ericoid fungi are present and whether these fungi are 'site-specific' at each commercial peat source. Additionally, further studies testing multiple peat products harvested from several strata are necessary to determine if an 'optimum' strata exists for the presence of ericoid mycorrhizae. Lastly, ericoid mycorrhizal fungi endemic to these commercially-harvested peat bogs need to be examined to determine if they have a host specificity and to what extent the mycorrhizal association benefits the growth and development of each ericaceous species under nursery and landscape conditions.

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