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Biology of the ectomycorrhizal genus, *Rhizopogon*

III. Influence of co-cultured conifer species on mycorrhizal specificity with the arbutoid hosts *Arctostaphylos uva-ursi* and *Arbutus menziesii*

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SUMMARY

Seedlings of *Pseudotsuga menziesii* (Mirb.) Franco, *Pinus ponderosa* Dougl. ex Laws, *Arbutus menziesii* Pursh., and cuttings of *Arctostaphylos uva-ursi* (L.) Spreng were grown in monoculture and in conifer-hardwood dual-culture combinations in the glasshouse and inoculated with spore slurries of six *Rhizopogon* species. The primary objectives were to assess and compare the pattern of host specificity between symbionts and to study the influence of co-cultured plants on ectomycorrhiza development. The *Rhizopogon* spp. ranged from genus-specific to multiple-host compatible. In monoculture, four *Rhizopogon* sp. (*R. ellenae* Smith, *R. occidentalis* Zeller & Dodge, *R. smithii* Hosford and *R. subcaerulescens* Smith) formed ectomycorrhizas with *Pinus ponderosa*, and two *Rhizopogon* sp. (*R. parksii* Smith and *R. vinicolor* Smith) formed ectomycorrhizas with *Pseudotsuga menziesii*. None of the fungi tested developed ectomycorrhizas on *Arbutus menziesii* or *Arctostaphylos uva-ursi* in monoculture. In dual culture, three of the four *Rhizopogon* species (*R. ellenae*, *R. occidentalis* and *R. subcaerulescens*) that formed ectomycorrhizas on *Pinus ponderosa*, formed some ectomycorrhizas on *Arbutus menziesii* and *Arctostaphylos uva-ursi*. *Rhizopogon parksii* and *R. vinicolor* only formed ectomycorrhizas on *Pseudotsuga menziesii*.

Key words: Compatibility, *Pseudotsuga menziesii*, *Pinus ponderosa*, madrone, bearberry.

INTRODUCTION

Rhizopogon is the largest genus of ectomycorrhizal hypogeous Basidiomycotina, with about 150 described species (Molina, Massicotte & Trappe, 1992). Species are most abundant in western North America where *Rhizopogon* associates with several Pinaceae hosts, especially *Pseudotsuga menziesii* (Douglas fir) and *Pinus* species. In pure-culture ectomycorrhizal syntheses, Molina & Trappe (1982*a*, 1994) described strong patterns of host specificity related to *Rhizopogon* genus sections. Several species showed specificity for either *Pseudotsuga* or *Pinus*, a pattern also seen in field collections of sporocarps, whereas others associated with other genera of Pinaceae.

Because the artificial nature of pure-culture syntheses creates problems in interpreting host-fungus compatibility (Duddridge, 1986*a*, *b*), Massicotte *et al*. (1994) tested the specificity hypotheses put forth by Molina & Trappe (1982*a*, 1994) for *Rhizopogon* by growing species of five genera of Pinaceae (*Abies*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga*) in pasteurized soil in pots and inoculating them with spores of 11 *Rhizopogon* spp. They grew the hosts in single- and two-host culture combinations to examine the effect of co-cultured plants on the ectomycorrhizal host range of the fungi. In single-host treatments, all *Rhizopogon* species developed ectomycorrhizas only on *Pseudotsuga menziesii* or *Pinus ponderosa* (pon-

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derosa pine); in two-host cultures, some *Rhizopogon* species maintained specificity for either *Pseudotsuga* or *Pinus*, whereas others also were able to colonize *Abies*, *Picea* or *Tsuga*. Massicotte *et al*. (1994) believed that those *Rhizopogon* species able to colonize *Abies*, *Picea* or *Tsuga* first formed ectomycorrhizas with *Pseudotsuga* or *Pinus* (primary hosts) and then spread vegetatively to the secondary hosts.

Results from mycorrhizal specificity studies are important from an ecological perspective, particularly in forests composed of different ectomycorrhizal hosts species, or when composition of host species changes during community succession. The ability of different plant species to associate with compatible ectomycorrhizal fungi can influence host establishment and the maintenance of fungal populations and species diversity during periods of change. For example, Perry *et al*. (1989), Molina *et al*. (1992), and Amaranthus & Perry (1994) describe the ecological contribution of pioneering hardwood shrubs and trees in the genera *Arbutus*, *Arctostaphylos*, *Castanopsis*, *Lithocarpus* and *Quercus* in maintaining ectomycorrhizal fungus diversity and activity for associations by late-seral Pinaceae. They emphasize the need to promote such natural associations in forest management and to avoid practices that reduce ectomycorrhizal fungus and host diversity.

Arbutoid hosts in the genera *Arbutus* and *Arctostaphylos* are important in this regard because they have the potential to associate with many known ectomycorrhizal fungi of forest trees. Zak (1976*a*, *b*) and Molina & Trappe (1982*b*) found that in pure-culture syntheses *Arbutus menziesii* (Pacific madrone) and *Arctostaphylos uva-ursi* (bearberry) were highly receptive to a wide range of ectomycorrhizal fungi, including several species assumed to be host-specific to Pinaceae. Molina *et al*. (1992, and unpublished) also found that in seedling bioassays of forest soils with mixtures of ectomycorrhizal hosts (*Pseudotsuga*, *Pinus*, *Abies*, *Lithocarpus* and *Arbutus* spp.), that Pacific madrone shared compatibility with fungi that were strongly specialized with either *Pseudotsuga* or *Pinus*, including some possible *Rhizopogon* associations. Given the beneficial effects of arbutoid hosts on establishment and growth of later successional Pinaceae (Danielson, 1984; Amaranthus & Perry, 1989; Borchers & Perry, 1990; Dahlberg, 1990; Horton, 1992; Visser, 1995) and the occurrence of Pacific madrone and *Arctostaphylos* spp. throughout much of the range of Pinaceae in western North America, it is important to document the shared compatibility for ectomycorrhizal fungi between arbutoid and ectomycorrhizal hosts.

We conducted a series of inoculation experiments following the methods of Massicotte *et al*. (1994). Two arbutoid and two ectomycorrhizal (Pinaceae) hosts were grown singly and in dual-host combinations and inoculated with six *Rhizopogon* species from different specificity groups. Our specific objectives were to explore the ectomycorrhizal potential of *Rhizopogon* species when inoculated via spores onto arbutoid hosts, and to examine the effects of co-cultured plants (*Pinus* and *Pseudotsuga*) on the compatibility of arbutoid hosts and *Rhizopogon* spp.

MATERIALS AND METHODS

Seedling and cutting preparation and growth conditions

Seeds of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and ponderosa pine (*Pinus ponderosa* Dougl.) were aerated for 15 h in Captan 50w® fungicide (4 ml l^{-1} of water) (Zeneca, Wilmington, DE, USA), placed in plastic bags with tissues moistened with Captan solution, and stored for 8 wk at 4 °C. Conifer seeds were then planted in Deepots® (500 ml capacity, 70 mm top diameter, 250 mm long) (Stuewe & Sons, Inc., Corvallis, OR, USA) containing an equal mixture by volume of peat and vermiculite, filled to 2·5 cm from the top of the container. Seeds were covered with white quartz sand (8 grade) to reduce splash during watering. Douglas fir and pine seeds germinated (*c*. 95%) within 5 ds.

Dried berries of Pacific madrone (*Arbutus menziesii* Pursh.) from Whidbey Island, Washington, were aerated in cold water for 20 h, aerated in Captan for 3 h, drained and placed in plastic bags with tissues moistened with Captan solution, and stored for 8 wk at 4 °C. Madrone berries were then macerated with a mortar and pestle, and planted in trays containing an equal mixture of peat and vermiculite and covered with white quartz sand. Madrone seeds germinated (*c*. 80%) within 6 wks and were transplanted into Deepot containers.

Bearberry (*Arctostaphylos uva-ursi* (L.) Spreng.) cuttings from mature plants growing outside the Forestry Sciences Laboratory, Corvallis, Oregon, were stored overnight in a plastic bag at 4 °C. Basal ends of 10-cm cuttings treated with Dip-n-Grow® rooting hormone (1% indolebutyric acid, 0·5% naphthaleneacetic acid) (Astoria-Pacific Inc., Clackamas, OR, USA), were inserted in propagation boxes filled with perlite and placed on a mist bed. Air temperature fluctuated between 20–22 °C and cuttings received 8 s of mist at 15-min intervals. Bearberry cuttings rooted (about 65%) in 10 wk and were transplanted into Deepot containers.

Plants were grown in the greenhouse under a combination of sunlight and artificial light (280 μmol m^{-2} s^{-1}) provided by sodium-vapour lamps. Air temperature fluctuated from 13 to 24 °C. Seeds were misted with tap water daily until germination, watered twice weekly until plants were about 12-wk-old, then watered weekly. Each pot received two 50-ml applications of Peter's® fertilizer

Table 1. *Fungal species, section affiliation, associated hosts, and collection location of* Rhizopogon *species*

Species	Associated hosts	Collection location (county, state)	Voucher number*
Rhizopogon parksii Smith	*Pseudotsuga menziesii* *Tsuga heterophylla*	Clallam Co., WA	T12050
Rhizopogon vinicolor Smith	*Pseudotsuga menziesii* *Pinus ponderosa*	Jefferson Co., OR	T12472
Rhizopogon ellenae Smith	*Pinus contorta* *Pinus muricatus*	Mendocino Co., CA	T11375
Rhizopogon subcaerulescens Smith	*Pseudotsuga menziesii* *Pinus* sp.	Jackson Co., OR	T12007
Rhizopogon occidentallis Zeller & Dodge	*Pinus contorta*	Coos Co., OR	No voucher†
Rhizopogon smithii Hosford	*Pinus contorta*	Coos Co., OR	T12006

* Voucher numbers of James M. Trappe. Specimens are deposited in the Oregon State University Herbarium (OSC).
† No voucher but same collection information as for T12006.

(N-P-K/473-449-426 ppm plus trace elements) applied full-strength 3 wk after transplanting and conifer germination, and half-strength at 7 months.

Inoculation and host combinations

Spore slurries of six *Rhizopogon* spp. (Table 1) were prepared according to Castellano & Molina (1989) from one or two sporocarps. Spore density was calculated by haematocytometry. Inoculum concentration (number of spores ml^{-1}) was standardized between the different fungi used by diluting the spore suspension with deionized water. Each species was applied at 500000 spores per pot over two inoculations except for *R. occidentalis* Zeller & Dodge, which was applied at 200000 spores per pot because of limited inoculum. For each inoculation, 10 ml of diluted spore suspension was pipetted onto the substrate. Plants were inoculated when they were 12- and 16-wk-old.

Nine or 10 replicate pots of Douglas fir, Pacific madrone, bearberry and combinations of Douglas fir with Pacific madrone and with bearberry were inoculated with *Rhizopogon parksii* Smith and *R. vinicolor* Smith; in previous studies (Massicotte *et al.*, 1994; Molina & Trappe, 1994), these two *Rhizopogon* spp. showed strong specificity for Douglas fir. Nine or ten replicate pots of ponderosa pine, Pacific madrone, bearberry and combinations of ponderosa pine with Pacific madrone or with bearberry were inoculated with *R. ellenae* Smith, *R. occidentalis*, *R. smithii* Hosford, and *R. subcaerulescens* Smith; in previous studies (Massicotte *et al.*, 1994; Molina & Trappe, 1994) these four *Rhizopogon* spp. showed mycorrhizal specialization towards *Pinus*. Eight to 10 pots of each host species served as non-inoculated controls to detect ectomycorrhizal fungus contaminants from the greenhouse.

Ectomycorrhiza assessment

Roots were examined when the plants were about 10 months old. For each plant, the number of ectomycorrhizal root tips of each inoculated fungus species were estimated visually with the aid of a dissecting microscope and the abundance classes were recorded as described in the next section. The presence and relative abundance of glasshouse ectomycorrhizal contaminants were noted but not analysed further.

Representative ectomycorrhizas were fixed and postfixed by procedures described by Massicotte, Ackerley & Peterson (1985) and Massicotte *et al.* (1986), rinsed and dehydrated in a graded ethanol series, and embedded in LR White resin (London Resin Company Ltd.). Sections (1·0–1·5 μm) were cut with glass knives and stained for light microscopy with 0·05 % toluidine blue O in 1 % sodium borate.

Statistical analyses

For each plant, the number of ectomycorrhizal root tips of the inoculated fungus species was converted into abundance classes as follows: 0 : 0 % colonization; Trace (T); 1–5 % colonization; 1 : 6–25 %; 2 : 26–50 %; 3 : 51–75 % and 4 : 76–100 %. Midpoints of the abundance classes were assigned for ranked nonparametric comparisons. Ranked midpoints of mycorrhizal-tip abundance were compared between hardwoods and conifers grown in dual culture for each inoculated fungus by a paired comparison of ranks (Iman, Hora & Canover, 1984). The host seedlings in an individual pot were considered a pair. A Bonferroni adjustment was applied to the level of significance based on the two comparisons made; comparisons were considered significantly different at $\alpha = 0{\cdot}05/\text{two comparisons}$ or $P \leqslant 0{\cdot}025$.

RESULTS

Ectomycorrhizal development

All six fungal species formed ectomycorrhizas on at least the conifer host (Table 2). In monocultures, the four *Pinus*-associated *Rhizopogon* spp., *R. ellenae*, *R. occidentalis*, *R. smithii*, and *R. subcaerulescens*, formed ectomycorrhizas with ponderosa pine and the two

Table 2. *Number of seedlings colonized by each fungus tested. Host species in parentheses indicate the alternate species present in dual host pots; n is the number of seedlings inoculated with a given fungus surviving to the end of the experiment**

Host	Co-cultured host present	*n*	Fungus					
			Rhizopogon smithii	*R. occidentalis*	*R. subcaerulescens*	*R. ellenae*	*R. parksii*	*R. vinicolor*
Ponderosa pine		10	10	9	10	8	—	—
Ponderosa pine	(Pacific madrone)	10	6	10	10	8	—	—
Ponderosa pine	(Bearberry)	10	0	8	9	8	—	—
Pacific madrone	(Ponderosa pine)	10	0	10	8	6	—	—
Bearberry	(Ponderosa pine)	9 or 10	0 (9)	4 (9)	8	6	—	—
Douglas fir		10	—	—	—	—	9	10
Douglas fir	(Pacific madrone)	10	—	—	—	—	10	10
Douglas fir	(Bearberry)	9 or 10	—	—	—	—	10	9 (9)
Pacific madrone	(Douglas fir)	10	—	—	—	—	0	0
Bearberry	(Douglas fir)	9 or 10	—	—	—	—	0	0 (9)
Pacific madrone		10	0	0	0	0	0	0
Bearberry		10	0	0	0	0	0	0

Number of seedlings in parentheses indicates $n < 10$.
* A dash line indicates that the fungal–host treatment was not performed.

Pseudotsuga-associated *Rhizopogon* spp., *R. parksii* and *R. vinicolor*, formed ectomycorrhizas with Douglas fir. None of the fungi formed ectomycorrhizas with either Pacific madrone or bearberry in monoculture.

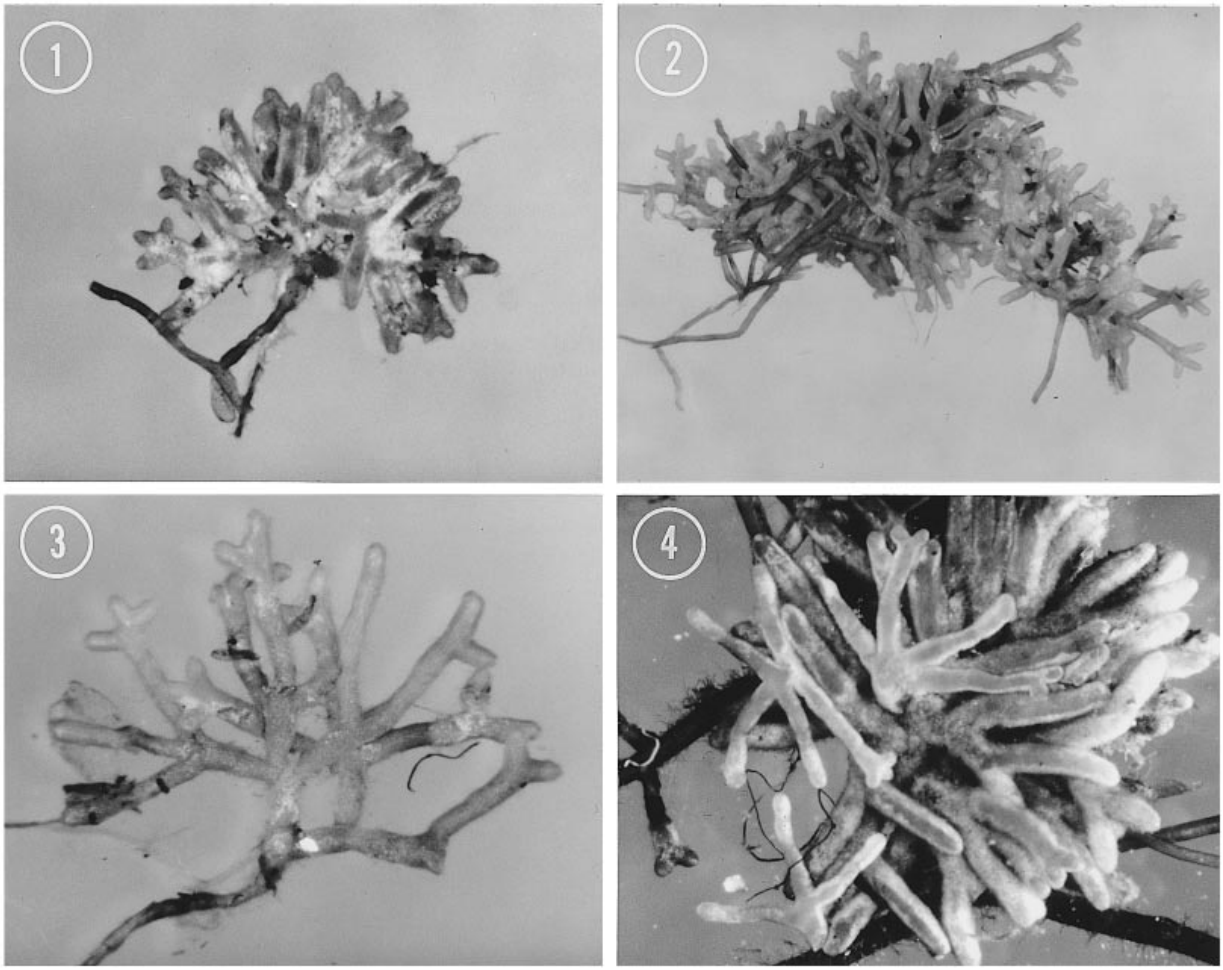
In dual cultures, specificity patterns differed among *Rhizopogon* spp. All six *Rhizopogon* spp. formed ectomycorrhizas with the conifer host (Table 2) although *R. smithii* formed ectomycorrhizas on ponderosa pine grown with Pacific madrone but not on ponderosa pine with bearberry. Three of the four *Pinus*-associated *Rhizopogon* spp., *R. ellenae*, *R. occidentalis*, and *R. subcaerulescens*, formed ectomycorrhizas with both Pacific madrone and bearberry. *Rhizopogon smithii* and the two *Pseudotsuga*-associated *Rhizopogon* spp., *R. parksii* and *R. vinicolor*, formed no ectomycorrhizas with either hardwood. Table 2 indicates the number of seedlings or cuttings colonized by each fungus.

Glasshouse ectomycorrhizal contaminants such as *Thelephora terrestris* and occasionally *Laccaria laccata* were regularly seen on all four hosts. Abundance was variable and ranged from absent or low to high for individual seedlings but was not quantified as for *Rhizopogon* inoculations. *Thelephora* colonization tended to be highest on uninoculated control conifer seedlings. We did not observe any relationship between the success of *Rhizopogon* inoculation and amount of *Thelephora* contamination present so we do not believe there was antagonism between fungi that affected overall results. The dark septate endophyte *Mycelium radicis atrovirens* was also sporadically observed.

Ectomycorrhiza characteristics

Characteristics of *Rhizopogon* mycorrhizas on ponderosa pine and Douglas fir were as described by Massicotte *et al*. (1994). On arbutoid hosts, colour, mantle texture, rhizomorphs and extending hyphae closely matched those on the neighbouring pine. Unlike the bifurcate branching patterns on pine, however, arbutoid mycorrhizas branched in typical cruciform patterns (Figs 1–4), often occurring as dense coralloid clusters; one large cluster formed by *R. occidentalis* on bearberry measured 12 mm across (Fig. 2). For individual *Rhizopogon* spp., mantle colour, texture, branching patterns and emanating hyphae were indistinguishable between the two arbutoid hosts.

Longitudinal sections of arbutoid mycorrhizas showed typical anatomy as described by Molina & Trappe (1982*b*) and Massicotte *et al*. (1993). All had characteristic para-epidermal Hartig nets and intracellular hyphal proliferation within epidermal cells. Mantle thickness, degree of Hartig net penetration and intracellular proliferation differed among the three *Rhizopogon* species. Mantles were most well developed and thickest with *R. subcaeru-*



Figures 1–4. Arbutoid mycorrhizas formed by three *Rhizopogon* spp. on bearberry. **Figure 1.** *R. ellenae* (× 13). **Figure 2.** *R. occidentalis* (× 5). **Figure 3.** *R. occidentalis* (× 13). **Figure 4.** *R. subcaerulescens* (× 13).

lescens, occasionally 10 or more hyphal layers thick (Figs 5–8). Mantles formed by *R. occidentalis* and *R. ellenae* were thinner than those formed by *R. subcaerulescens* and with loose arrangements of surface hyphae. Hartig nets formed by *R. occidentalis* and *R. subcaerulescens* were well developed, typically penetrating through the epidermal layer and occasionally surrounding an epidermal cell (Figs 5, 7); Hartig nets formed by *R. ellenae* were wedge shaped, only partially penetrating into the epidermal layer. Similarly, intracellular hyphae of *R. occidentalis* and *R. subcaerulescens* were well developed, often completely filling the epidermal cell (Figs 5–8), whereas *R. ellenae* ramified loosely within epidermal cells.

Anatomical structure differed little between arbutoid hosts for individual fungi with the exception of *Rhizopogon occidentalis*. On Pacific madrone, *R. occidentalis* displayed a stronger presence of large-diameter hyphae in the mantle and Hartig net than on bearberry.

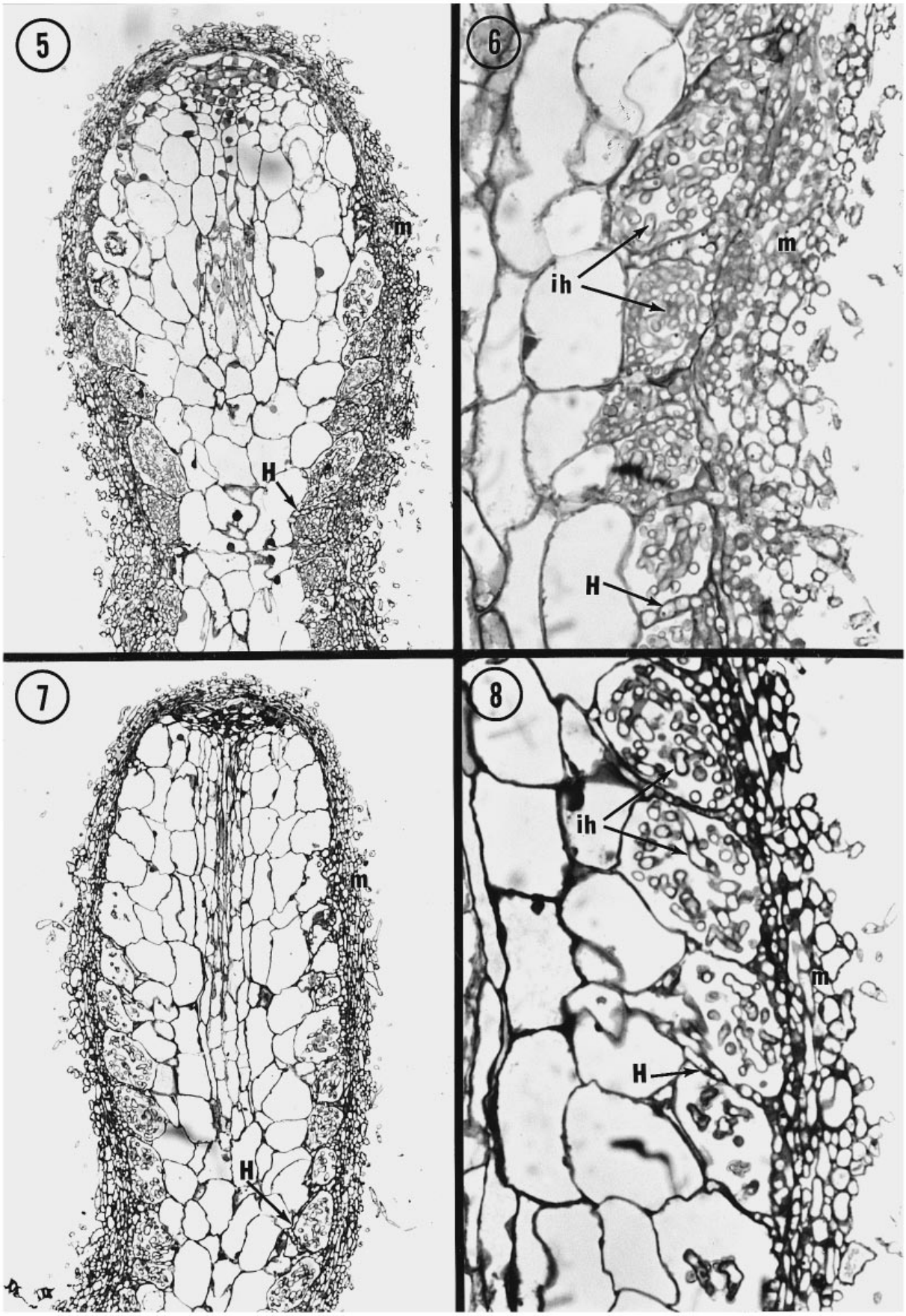
Rhizopogon vinicolor and *R. parksii* formed abundant, well-developed ectomycorrhizas on Douglas fir as described by Massicotte *et al*. (1994). Abundant emergent hyphae and rhizomorphs permeated the rooting substrate in single and dual-host cultures. Both fungal species grew along the surface of Pacific madrone and bearberry roots, and occasional dark, patch like crusts of mycelium, similar in colour to Douglas-fir mantles, occurred on lateral feeder roots. Freezing microtome sections of these colonized roots, however, revealed only a patchy mantle structure and occasional wedge like protrusions between epidermal cells. True Hartig nets or intracellular hyphal penetration were not observed.

Abundance of colonization

The amount of colonization by *R. ellenae* did not differ between ponderosa pine and the hardwood species. By contrast, the abundance of colonization by *R. occidentalis* and by *R. subcaerulescens* was greater on ponderosa pine than on Pacific madrone but did not differ between ponderosa pine and bearberry (Fig. 9). There was no colonization of the hardwood species grown in monoculture.

DISCUSSION

These results strongly support previous studies on specificity of *Rhizopogon* spp. with species of Pinaceae. In monoculture, *Rhizopogon* spp. developed



Figures 5–8. Longitudinal sections of arbutoid mycorrhizas formed by *Rhizopogon subcaerulescens* on bearberry and Pacific madrone. H, Hartig net; ih, intracellular hyphae; m, mantle. **Figure 5.** *R. subcaerulescens* + bearberry (× 185). **Figure 6.** *R. subcaerulescens* + bearberry (× 528). **Figure 7.** *R. subcaerulescens* + Pacific madrone (× 210). **Figure 8.** *R. subcaerulescens* + Pacific madrone (× 528).

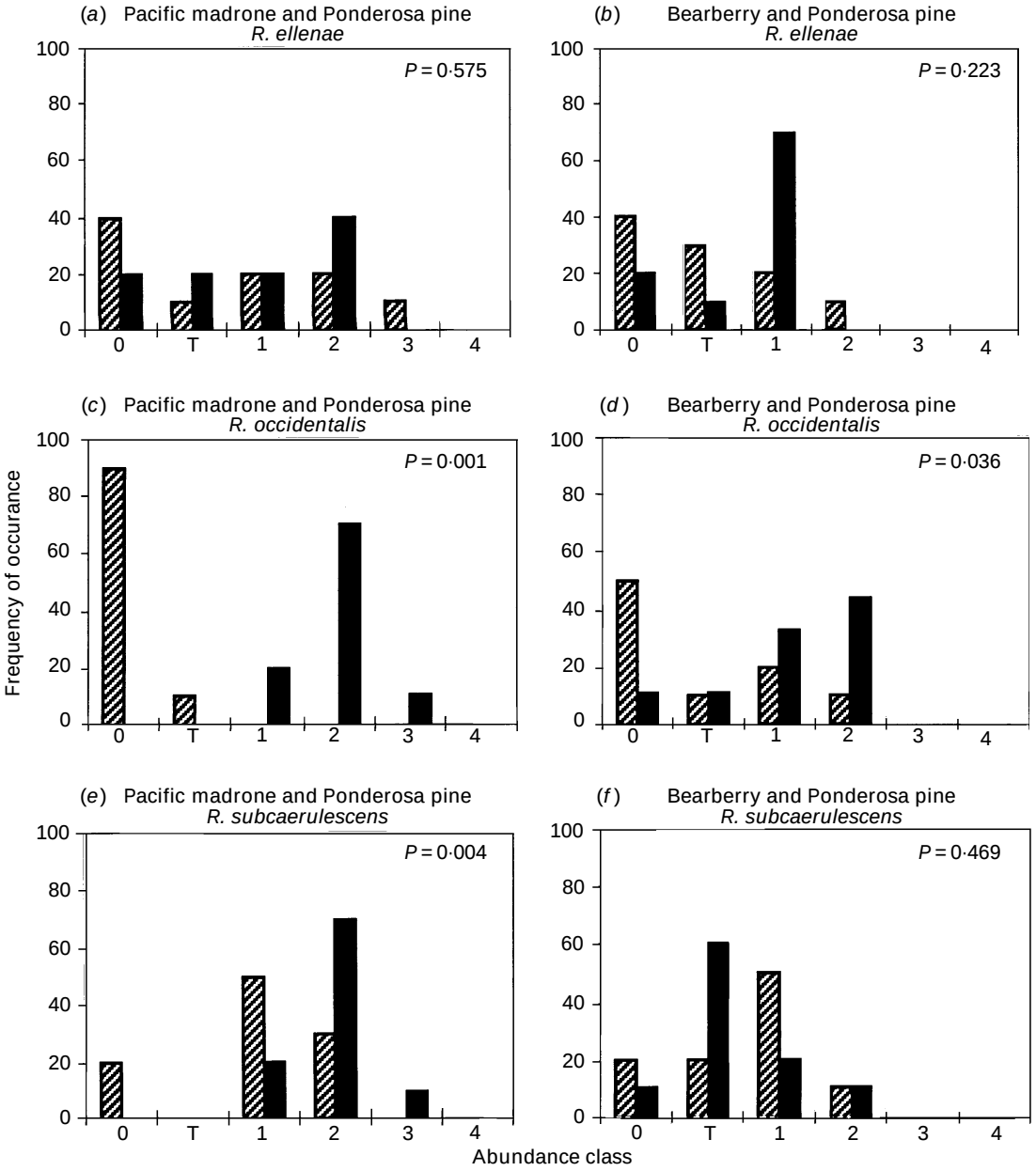


Figure 9. Frequency distribution of abundance classes of *Rhizopozon* mycorrhizas on pine and hardwood seedlings ($n = 9$ or 10) grown in dual culture. P-values obtained from a Kruskal–Wallis test of difference in abundance between hosts are significant if $P \leqslant 0{\cdot}025$, after Bonferroni adjustment. ▨, ectomycorrhizal colonization of hardwoods; ■, ectomycorrhizal colonization of conifers.

ectomycorrhizas only on Douglas fir or ponderosa pine. As seen in Massicotte *et al*. (1994), however, in dual-host culture, three *Rhizopogon* species that colonized ponderosa pine (*R. ellenae*, *R. occidentalis* and *R. subcaerulescens*) formed ectomycorrhizas on adjacent arbutoid hosts. We agree with the original hypothesis of Massicotte *et al*. (1994) that spores of *Rhizopogon* species germinated in the rhizosphere of pine seedlings, developed ectomycorrhizas with pine, and then spread vegetatively to colonize adjacent arbutoid host roots. It is also possible, however, that pine roots stimulated spore germination and that these germinating spores initiated arbutoid mycorrhizal development on bearberry and Pacific madrone. These possibilities indicate a need to investigate experimentally host–fungus recognition factors that operate during spore germination as well as the contact of vegetative mycelium with root surfaces.

Our results also support the patterns of host specificity found by Molina & Trappe (1982*a*, 1994) and Massicotte *et al*. (1994). The two Douglas-fir-specific fungi, *R. vinicolor* and *R. parksii*, did not spread onto the arbutoid hosts, even though abundant mycelium and rhizomorphs permeated the rooting substrate and contacted arbutoid host roots;

some surface colonization and patch like mantles occasionally were noted on arbutoid host roots, but we did not observe Hartig net development or intracellular hyphal penetration. Massicotte *et al*. (1994) also found that these two species were unable to develop ectomycorrhizas on *Picea*, *Tsuga* or *Abies* species when grown in dual culture with Douglas-fir. Zak (1976*a*) and Molina & Trappe (1982*b*), however, were able to synthesize arbutoid mycorrhizas on bearberry and Pacific madrone with *R. vinicolor* and *R. parksii* in pure-culture syntheses. These contrasting results emphasize the need to evaluate specificity patterns under natural soil conditions.

Our results with the pine isolates of *Rhizopogon* also mirrored results from Massicotte *et al*. (1994). *Rhizopogon* spp. that showed the highest specificity for *Pinus* association in that study showed low levels (*R. occidentalis*) or no colonization (*R. smithii*) on the co-cultured arbutoid hosts. *Rhizopogon ellenae* and *R. subcaerulescens*, on the other hand, were able to spread from *Pinus* to other Pinaceae genera and likewise showed the highest frequency and abundance of mycorrhizal formation with the two arbutoid hosts in co-culture with *Pinus*. Overall, these results confirm the three specificity-groupings for the genus *Rhizopogon* put forth by Molina & Trappe (1994) and Massicotte *et al*. (1994): Douglas-fir specialists, pine specialists and a broadly Pinaceae-associated group.

Zak (1974) observed that field collections of Pacific madrone mycorrhizas appeared similar to ectomycorrhizas of neighbouring Douglas fir and grand fir in overall colour, mantle structure and emanating hyphae, and concluded that many of the ectomycorrhizal fungi of conifers probably formed ectendomycorrhizas (arbutoid mycorrhizas) on Pacific madrone, a factor he later demonstrated in pure-culture syntheses (Zak, 1976*a*, *b*). Molina & Trappe (1982*b*) expanded on Zak's work and synthesized 25 ectomycorrhizal fungal species on bearberry and Pacific madrone, including species that typically associate with specific Pinaceae genera. They hypothesized that arbutoid hosts show a broad receptivity towards ectomycorrhizal fungi, broader than seen with most ectomycorrhizal hosts. Results from the present study support this hypothesis, and all mycorrhizas showed a similarity in external ectomycorrhizal characters on both Pinaceae and arbutoid hosts. Three *Rhizopogon* spp., considered Pinaceae associates, were able to form well developed arbutoid mycorrhizas on both bearberry and Pacific madrone. By contrast, by using the method reported in this study, we have found these same *Rhizopogon* spp. unable to colonize two other hardwood ectomycorrhizal species, *Lithocarpus densiflorus* (tanoak) and *Castanopsis chrysophylla* (chinkapin), in dual culture with ponderosa pine. In a mixed-seedling species bioassay of south-western Oregon forest soils, Molina *et al*. (1992, and unpublished) also found that Pacific madrone developed arbutoid mycorrhiza with one *Rhizopogon* spp. and two other fungi that otherwise only associated with ponderosa pine; in the same study, tanoak did not associate with these same fungi.

Hardwoods such as Pacific madrone, manzanita (*Arctostaphylos*), tanoak and chinkapin are common early seral hardwood shrubs and trees that quickly occupy disturbed forest sites in southwestern Oregon and often remain throughout the life of the stand. Given the regularity of disturbance in these ecosystems, particularly by fire or clear-cutting, Perry *et al*. (1989) and Molina *et al*. (1992) emphasize the importance of these pioneering species in maintaining ectomycorrhizal diversity in these forest ecosystems. The ability of arbutoid hosts in the genera *Arbutus* and *Arctostaphylos* to develop mycorrhizas with fungi commonly associated with a variety of Pinaceae spp. (Molina & Trappe 1982*b*) might be particularly important in this regard. Amaranthus & Perry (1989) and Borchers & Perry (1990) demonstrated the strong influence of Pacific madrone in enhancing ectomycorrhizal diversity of Douglas-fir seedlings by comparing seedlings planted in soil taken from beneath Pacific madrone with soil from beneath other ectomycorrhizal hosts or from recent forest clearings. In coastal mixed-conifer and chaparral communities of north-central California, Horton (1992) notes that Douglas fir invades almost exclusively into patches of *Arctostaphylos* dominated chaparral rather than in adjacent *Adenostoma fasciculatum* – dominated chaparral; *A. fasciculatum* forms vesicular–arbuscular mycorrhizas. He hypothesized that the mycorrhizal fungus associates of *Arctostaphylos* provide ectomycorrhizal fungus inoculum for Douglas-fir, thereby facilitating survival of Douglas-fir seedlings and eventually yielding forest establishment in *Arctostaphylos* dominated chaparral. He has subsequently used molecular techniques to confirm the sharing of compatible ectomycorrhizal fungi between Douglas-fir and *Arctostaphylos* on those sites (pers. comm.). Danielson (1984), Visser (1995), and Dahlberg (1990) have noted a similar role for *Arctostaphylos uva-ursi* as an understory ectomycorrhizal component in northern temperate forest ecosystems. Thus arbutoid plants are important guild hosts in sustaining diversity by maintaining ectomycorrhizal fungus inoculum after disturbance, thereby influencing the succession of the forest towards later seral dominance by Pinaceae.

By using molecular techniques, Cullings, Szaro & Bruns (1996) found that some mycotrophic, achlorophyllos species in the Monotropoideae (Ericaceae) preferentially form mycorrhizal associations with suilloid fungi, a monophyletic group that includes *Rhizopogon* and *Suillus* (Bruns *et al*., 1989; Bruns & Szaro, 1992). Sporocarps of *Rhizopogon* and *Suillus* are restricted to Pinaceae species in the field (Molina

et al., 1992). Cullings *et al*. (1996) found that one monotrope species, *Pterospora andromedea*, was highly restricted in fungus associates; all 31 individuals examined from field collections associated exclusively with the *R. subcaerulescens* species group. In our study, *R. subcaerulescens* showed the strongest development with both bearberry and Pacific madrone. Given the close evolutionary connections between Monotropoideae and *Arctostaphylos* (Cullings, 1992; Cullings *et al*., 1996), it is important to note the similarity for mycorrhizal association with Pinaceae-restricted mycorrhizal associates such as *Rhizopogon*. Such a strongly connected guild structure (Pinaceae overstory – Pinaceae ectomycorrhizal fungus specialists – understory Monotropoideae) emphasizes the important role mycorrhizal specificity can play in forest community organization (Molina *et al*., 1992; Cullings *et al*., 1996). For example, in the case of *Pterospora andromeda*, *R. subcaerulescens* would need to be present on Pinaceae hosts in order for *P. andromeda* to occur in the understory of that particular forest stand.

Further research is needed to follow-up these investigations. First, what types of recognition phenomena are operating that allow arbutoid hosts to associate with a diverse range of ectomycorrhizal fungi, particularly those otherwise considered host-specific at least in sporocarp association, with the Pinaceae, such as the genera *Rhizopogon* and *Suillus*? Recognition factors between ectomycorrhizal fungi and hosts are poorly understood and little explored. Given the unique attribute of intracellular penetration of epidermal cells that distinguishes arbutoid anatomy from typical ectomycorrhizal anatomy and this range in fungal associates, arbutoid hosts are ideal plants to investigate in this regard. Differences in cell wall structure and physiology (e.g. production of enzymes and other metabolites) could provide important clues to understanding mechanisms of host–fungus recognition and maintenance of the symbiotic structure in ectomycorrhizas. Secondly, if Pinaceae-specific fungi associate with arbutoid hosts in the field, as this glasshouse study and other field studies suggest (Amaranthus & Perry 1989; Borchers & Perry, 1990; Horton, 1992), then do the arbutoid hosts maintain these fungi in active mycorrhizal associations once the neighboring Pinaceae hosts are removed by disturbance? One approach to addressing these questions is to conduct dual-host experiments similar to those described in this study wherein the associated pine seedling is decapitated and then after 1–2 years examine whether *Rhizopogon* maintains mycorrhizas on the arbutoid host. The use of molecular probes or DNA sequencing specific to *Rhizopogon* and other suilloid fungi, as used by Cullings *et al*. (1996), on field collected roots of *Arbutus* and *Arctostaphylos* mycorrhizas in the absence of nearby Pinaceae, is another approach to exploring this phenomenon.

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