Summary We studied the effects of broad-spectrum light quality on the interaction between the ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker and Couch and Scots pine (*Pinus sylvestris* L.) seedlings and hypocotyl cuttings cultured in vitro. The light sources were cool white (CW), warm white (WW) and red-rich daylight (RD) fluorescent lamps. Inoculation with *P. tinctorius* enhanced adventitious root formation of the cuttings in all light treatments. Rooting of the inoculated cuttings was highest in WW light (89%), followed by CW (73%) and RD light (66%). During 6 weeks of in vitro culture, rooted cuttings formed only a few lateral roots. The fungus grew over lateral roots, but the Hartig net was absent in all light treatments. In non-inoculated cuttings, neither root formation nor subsequent root growth was affected by light quality. In the seedling experiment, inoculation in the WW treatment resulted in a significantly (*P* < 0.05) greater number of lateral roots than inoculation in the RD treatment. The percentage of lateral roots covered with fungal hyphae was also highest in WW light (62%), followed by CW (50%) and RD (27%) light. A similar pattern was observed in the intensity of Hartig net formation. We conclude that effects of broad-spectrum light quality on the ectomycorrhizal fungus–root interaction are dependent on the developmental stage of the root.

Keywords: adventitious rooting, cool white light, daylight, *Pinus sylvestris*, *Pisolithus tinctorius*, warm white light.

Introduction

Light modifies plant growth and development through various signal transduction networks. The photosensory system (for recent reviews, see Lin 2002, Quail 2002) encompasses three known classes of informational photoreceptors: cryptochromes and phototropins, which monitor the blue (390–500 nm) and ultraviolet-A (320–390 nm) regions of the spectrum, and the phytochromes, which mainly monitor the red (R) (600–700 nm) and far-red (FR) (700–750 nm) regions of the solar spectrum. The combined activities of these light receptors followed by signal transduction and integration, enable plants to detect and respond to differences in light flux, photoperiod, spectral composition and directionality.

Ectomycorrhizas (ECMs) are symbiotes formed between certain fungal and plant species in which the fungal partner provides amino acids, nutrients and water to a host plant in exchange for photosynthates (Smith and Read 1997). Because the fungal partner acts as a carbohydrate sink, thereby altering both photosynthesis and carbohydrate allocation in its host plant (Nehls and Hampp 2000, Wright et al. 2000), changes in light flux (Ekwebelam and Reid 1983, McGee and Furby 1992) and spectral quality (de la Rosa et al. 1998, 1999) can affect mycorrhizal interactions. De la Rosa et al. (1998) studied the growth of fertilized Scots pine (*Pinus sylvestris* L.) seedlings under different broad-spectrum lights and found that light with a low R:FR ratio reduced the number of short roots and developing mycorrhizas, which the authors proposed was a result of decreased dry mass allocation to roots. However, no effect of supplementary FR light on mycorrhizal formation or morphotype frequencies was observed in unfertilized seedlings (de la Rosa et al. 1999).

There have been attempts to promote adventitious rooting by ECM inoculation. Inoculation with specific ECM fungi can increase rooting frequency and the number of adventitious roots per shoot (e.g., Gay 1990, Normand et al. 1996, Karabaghi et al. 1998, Niemi et al. 2000). In previous in vitro studies performed in broad-spectrum cool white light, we found that the ECM fungus *Pisolithus tinctorius* (Pers.) Coker and Couch enhanced rooting and subsequent growth of Scots pine hypocotyl cuttings and formed mycorrhizas with Scots pine seedlings (Niemi et al. 2002a, 2002b). In the present study, we investigated whether broad-spectrum light quality affects the Scots pine–*P. tinctorius* interaction during root and mycorrhiza formation in vitro.
Materials and methods

Plant material

Scots pine seeds from open-pollinated plants, originating from Konginkangas in central Finland (63° N, 26° E), were surface-sterilized with 2% calcium hypochlorite for 20 min, rinsed in sterile water and germinated in vitro on 0.7% water agar in glass jars. The germinating seeds were incubated in darkness for 3 days and then transferred to a growth chamber with a temperature of 25 ± 2 °C and a 16-h photoperiod. Photosynthetic photon flux (PPF) was 140–150 µmol m⁻² s⁻¹ (from cool white (CW) fluorescent lamps; Airam L36W-642, Airam, Helsinki, Finland) (Figure 1). Hypocotyl cuttings used in the rooting experiment were prepared from 17-day-old seedlings by cutting the stem 5 mm above the root collar. In the experiment on mycorrhiza formation of seedlings, plant material of the same age and origin as that for hypocotyl cuttings was used.

Fungal material

The ECM fungus *P. tinctorius* was originally isolated under a Scots pine stand in Sweden (Strandberg-Arveby 1980), and deposited in the culture collection at the Swedish University of Agricultural Sciences, Uppsala, Sweden (strain 1984). The *P. tinctorius* strain was identified at the DNA-level by Sims et al. (1999). We have shown previously that the fungus is able to form mycorrhizas with Scots pine (Niemi et al. 2002a, 2002b). For this study, the fungus was maintained by cultivating the mycelium on Melin-Norkrans (MMN1) agar medium (pH 5.8) (Marx 1969), as modified by Heinonen-Tanski and Holopainen (1991). Four-week-old fungal mycelia were used for both cutting and seedling experiments.

Light spectral composition, fungal inoculation and rooting of hypocotyl cuttings in vitro

For rooting, 9-cm diameter petri dishes were filled with 25 ml of MMN2 rooting medium (pH 5.7) (Marx 1969) as modified by Niemi et al. (2002b). The surface of the agar was covered with a sterile moist filter paper. An individual cutting was laid horizontally on the filter paper and inoculated with a mycelial plug, 5 mm in diameter and cut from the edge of the fungal culture, close to the base of the cutting. In the control cultures, an agar plug was substituted for the mycelial plug. To prevent desiccation, the fungus and the base of the cutting were covered with a semicircular piece of brown paper to protect the fungus and developing root system from direct light. The shoot was unshaded.

The petri dishes were incubated in the growth chamber at a 70° angle to the vertical in a 16-h photoperiod with one of the following three broad-spectrum light sources, which, based on their spectra, were designated cool white (CW) (Airam L36W-642), warm white (WW) (Osrarn L36-W/30, Osrarn, Munich, Germany) and red-rich daylight (RD) (Nature de Luxe Osram L36-W/76). The spectral characteristics of the light sources are shown in Figure 1. For all light sources, PPF was 140–150 µmol m⁻² s⁻¹. The experiment comprised 25–35 cuttings per treatment, and was repeated twice.

The cuttings were rooted for 6 weeks. At harvest, the number of rooted cuttings was recorded. The length and fresh mass of the adventitious roots and the number of lateral roots were determined. The length of the primary needles and shoot fresh and dry masses (70 °C for 24 h) were also determined for rooted cuttings. Dry mass of the roots of cuttings was not determined because the non-inoculated roots had a low fresh mass. The percentages of shoot dry mass were 29.3, 26.4 and 27.7 for non-inoculated cuttings and 29.5, 28.4 and 29.1 for inoculated cuttings in the CW, WW and RD treatments, respectively, and did not differ significantly (P > 0.05) among fungal or light treatments. Therefore, shoot:root ratios for cuttings were determined based on fresh masses. The number of lateral roots covered with fungal hyphae was determined with the aid of a dissecting microscope and mycorrhizal structures were examined by light microscopy.

Light spectral composition and growth and mycorrhiza formation of inoculated seedlings in vitro

For the seedling experiment, 9-cm diameter petri dishes were filled with 25 ml of MMN2 agar medium (pH 5.7). The surface of the agar was covered with a sterile moist filter paper and an individual seedling was placed horizontally on the filter paper. All seedlings were inoculated with *P. tinctorius* by placing a mycelial agar plug close to the root system. The seedlings were cultivated under the same conditions as the cuttings in the rooting experiment. The experiment consisted of 25–26 seedlings per treatment, and was repeated twice.

The seedlings were cultivated for 8 weeks. At harvest, the length and fresh mass of the roots, number of lateral roots and shoot fresh mass were determined. The number of lateral roots covered with fungal hyphae was determined with the aid of a dissecting microscope and mycorrhizal structures were assessed by light microscopy.

Light microscopy

Longitudinal sections of lateral roots covered with fungal hyphae were prefixed in 0.1 M phosphate buffer (pH 7.0) containing 2.5% glutaraldehyde for 1 day and then postfixed for 4 h in 1% osmium tetroxide and dehydrated in a graded ethanol series. The root samples were infiltrated and embedded in Ladd’s LX 112 resin. The sections were cut with an LKB III...
Ultratome and stained with Toluidine blue (Merck, Whitehouse Station, NJ). Observations of the root sections were made with a Nikon Microphot FXA (Nikon, Tokyo, Japan) light microscope.

**Light spectral composition and the radial growth of Pisolithus tinctorius**

A 5-mm diameter mycelial plug was cut from the edge of a 1-month-old fungal colony and placed in the center of the MMN2 agar medium. The lid of the petri dish was covered with a white filter paper and the dishes were placed in CW, WW or RD light. The fungi were cultured for 4 weeks, and growth of the mycelium from the edge of the agar plug was determined weekly. Each treatment contained 10 replicates.

**Statistical analyses**

The percentage of cuttings with roots and the percentage of lateral roots of seedlings and cuttings covered with fungal hyphae were analyzed by the $\chi^2$ test with Bonferroni correction (Zar 1984, Altman 1991). In the seedling experiment, differences in growth parameters were compared by analysis of variance (ANOVA) and Tukey’s HSD. When the data for seedlings were not distributed normally, a non-parametric Kruskal-Wallis test was used, combined with Mann-Whitney U-test with Bonferroni correction. The same non-parametric test was used to compare the effects of light source on cutting growth parameters and radial growth of the fungus. No significant ($P > 0.05$) differences were found between duplicate experiments, and therefore, the data from the separate experiments were pooled. All statistical analyses were conducted with SPSS/PC (Version 9.0, SPSS, Chicago, IL).

**Results**

**Rooting of hypocotyl cuttings**

Inoculation with *P. tinctorius* increased the percentage of cuttings with roots in all light treatments (Table 1). Inoculated cuttings rooted significantly ($P < 0.05$) better in WW light than in RD light, whereas root formation of the non-inoculated cuttings was unaffected by light source (Table 1).

Adventitious roots were longer ($P < 0.05$) on cuttings inoculated with *P. tinctorius* than on non-inoculated cuttings grown in WW or RD light (Figure 2A). The number of lateral roots was low in both non-inoculated and inoculated cuttings (data not shown). However, the number of lateral roots increased significantly in response to inoculation in all light treatments (Figure 2B). Root fresh mass (Figure 2C), primary needle

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**Table 1. Inoculation with the ectomycorrhizal fungus Pisolithus tinctorius and light sources with different spectral quality and rooting of Scots pine hypocotyl cuttings in vitro. Cuttings were rooted for 6 weeks on MMN2 rooting medium. Within a column, different letters denote significant ($P < 0.05$) differences between the rooting percentages according to $\chi^2$ test with Bonferroni correction; $n = 51–60$. Abbreviations: CW = cool white light; WW = warm white light; and RD = red-rich daylight.**

<table>
<thead>
<tr>
<th>Light source</th>
<th>Non-inoculated</th>
<th>Inoculated</th>
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<tbody>
<tr>
<td>CC</td>
<td>43.3 a</td>
<td>73.1 ab</td>
</tr>
<tr>
<td>WW</td>
<td>36.7 a</td>
<td>89.0 a</td>
</tr>
<tr>
<td>RD</td>
<td>36.1 a</td>
<td>66.7 b</td>
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**Figure 2. Inoculation with the ectomycorrhizal fungus Pisolithus tinctorius, light spectral quality and growth of rooted Scots pine hypocotyl cuttings. Cuttings were rooted for 6 weeks on MMN2 rooting medium. Values are means ± SE ($n = 22–47$, number of rooted cuttings). Different letters above the bars within a light source denote significant ($P < 0.05$) differences between means according to Mann-Whitney U-test. Light sources: CW = cool white light; WW = warm white light; and RD = red-rich daylight.**
length (Figure 2D) and the root:shoot ratio (Figure 2E) of the cuttings were also increased by inoculation with *P. tinctorius* in all light treatments. The light treatments had no significant effect on growth of the inoculated cuttings. *Pisolithus tinctorius* covered 57, 45 and 40% of the lateral roots in the CW, WW and RD treatments, respectively. However, no Hartig net formation in the cutting roots was observed after the 6-week rooting period. Control cuttings grew slightly better in the CW and RD treatments than in the WW treatment (Figures 2A–E), but as with inoculated cuttings, differences among light treatments were not significant.

**Growth and mycorrhiza formation of seedlings**

Seedlings inoculated with *P. tinctorius* formed significantly (*P* < 0.05) more lateral roots in the WW treatment than in the RD treatment, but other root parameters were unaffected by the light treatments (Table 2). The number of lateral roots covered with fungal hyphae was highest in the WW treatment, followed by the CW and RD treatments (Table 3). The same pattern was observed in the intensity of Hartig net formation. Fungal hyphae reached the intercellular space of the cortex in seedlings growing in WW light only (Table 3).

**Radial growth of the fungus**

The concentration of glucose in the MMN2 rooting medium was low (1.1 mM) and, therefore, *P. tinctorius* grew slowly in the absence of a cutting or seedling (Figure 3). After four weeks in culture, the radial growth of the fungus in WW light was significantly (*P* < 0.05) higher than in CW light.

**Discussion**

Plants adapt to fluctuations in light quality during the day and year, and during changes in plant community (Cosgrove 1994, Smith 1994, Aphalo and Ballare 1995). Furthermore, populations growing at different latitudes may differ in their light requirements over longer periods (Clapham et al. 1998, 2002). Broad-spectrum CW light is often used in vitro studies of root and mycorrhiza formation. In the present in vitro study, we investigated whether broad-spectrum light quality influences the ECM fungus–Scots pine interaction during root and mycorrhiza formation.

Irrespective of light quality, inoculation with *P. tinctorius* increased formation and growth of roots on Scots pine hypocotyl cuttings in vitro. This result is consistent with our previous findings (Niemi et al. 2002a, 2002b) and corroborates studies with hypocotyl cuttings of different pine species (Gay 1990, Normand et al. 1996). Light sources differing in spectral distributions had no effect on control cuttings, but altered the fungus–cutting and fungus–seedling interactions. Red-rich daylight, which had the highest content of red and blue light, inhibited fungal-induced rooting of the cuttings, as well as lateral root and mycorrhiza formation on the seedlings, but had no effect on root elongation or root fresh mass, indicating that light-quality-mediated reactions by inoculated cuttings and seedlings, and their effects on ECM formation, are strongly affected by light quality.
Light spectral quality affects both photosynthetic rate and carbohydrate partitioning. Red-rich light has been shown to enhance leaf carbohydrate concentrations (Holzapfel et al. 1983, Britz et al. 1985, Saebø et al. 1995a). It has been suggested that the inhibition of rooting by red-rich light compared with blue-rich and CW lights is a result of reduced carbohydrate transport to the shoot base (Saebø et al. 1995b). De la Rosa et al. (1998) found that a decrease in the R:FR ratio from 1.30 to 0.39 reduced dry mass allocation to roots and mycorrhiza formation, but in fertilized plants only. Ratios of R:FR ratios were much higher in this study than in natural daylight (1.05–1.25) (Smith 1994). Warm white light, with an intermediate R:FR (10.4), enhanced the plant–fungus interaction compared with RD light with the highest R:FR (43.1) and CW light with the lowest R:FR (6.8). Similar shoot:root ratios (fresh mass) and percentages of shoot dry masses in the inoculated cuttings indicated that long-term changes in the amount and quality of R light did not greatly affect photosynthesis or carbohydrate allocation.

It has been suggested that phytochrome regulates transport of plant growth regulators, including auxin (Tian and Reed 2001, Halliday and Frankhauser 2003). In studies with auxin-treated in vitro cuttings (Bielenin 2000) and in vitro shoots (Gabryszewska and Rudnicki 1997), red light has resulted in similar or better rooting than blue or white lights. However, in the present study, long-term exposure to RD light disturbed both fungal-induced rooting of cuttings and lateral root and mycorrhiza formation by the seedlings. These processes have been reported to be under the regulation of indole-3-acetic acid (IAA) produced by ECM fungi (Karabaghli et al. 1998, Karabaghi-Degron et al. 1998, Kaska et al. 1999, Tranvan et al. 2000, Laurans et al. 2001). We did not measure IAA production by the fungus. However, the P. tineovarius strain used in this study produces considerable amounts of IAA in vitro, and improved rooting caused by this strain seems to be at least partly associated with its IAA production (Niemi et al. 2002b).

In conclusion, broad-spectrum CW light is most often used during in vitro rooting and mycorrhiza formation in growth chambers. Although WW and RD light did not affect the rooting ability of non-inoculated Scots pine cuttings, these light sources altered the interaction between Scots pine and P. tineovarius compared with CW light. It is likely that these changes are associated with compounds essential for the symbiotic interaction. Roots of the inoculated seedlings and cuttings reacted differently to the three light sources, indicating that the reactions were highly dependent on the developmental stage of the root.

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