Mycorrhiza (2008) 18:393–401
DOI 10.1007/s00572-008-0193-2

ORIGINAL PAPER

Root hydraulic properties and growth of balsam poplar (*Populus balsamifera*) mycorrhizal with *Hebeloma crustuliniforme* and *Wilcoxina mikolae* var. *mikolae*

J. Aurea Siemens · Janusz J. Zwiazek

Received: 12 May 2008 / Accepted: 17 July 2008 / Published online: 7 August 2008
© Springer-Verlag 2008

Abstract The effects of an E-strain fungus (*Wilcoxina mikolae* var. *mikolae*) and an ectomycorrhizal fungus (*Hebeloma crustuliniforme*) on growth and water relations of balsam poplar were examined and compared in the present study. Balsam poplar roots inoculated with *W. mikolae* var. *mikolae* (Wm) exhibited structures consistent with ectendomycorrhizal (EEM) associations, including a mantle surrounding the outside of the root and an extensive Hartig net that was located between cortical cells and extended to the vascular cylinder. Roots colonized with *H. crustuliniforme* (Hc) developed a mantle layer, indicative of an ectomycorrhizal (ECM) association, around the outer part of the root, but no distinct Hartig net was present. Wm-colonized balsam poplar also showed increased shoot growth, stomatal conductance (g_s), and root volumes compared with non-inoculated and Hc-inoculated plants. However, Hc-inoculated plants had higher root hydraulic conductivity (L_{pr}) compared with non-inoculated plants and Wm-inoculated plants. These results suggest that L_{pr} was not a growth-limiting factor in balsam poplar and that hyphal penetration of the root cortex in itself may have little influence on root hydraulic properties.

Keywords Ectomycorrhizal (ECM) fungi · Ectendomycorrhizal (EEM) fungi · Root hydraulic conductivity (L_{pr}) · Transpiration rate · Root–water relations

J. A. Siemens · J. J. Zwiazek (✉)
Deptartment of Renewable Resources, University of Alberta,
4-42 Earth Sciences Bldg.,
Edmonton, AB T6G 2E3, Canada
e-mail: janusz.zwiazek@ualberta.ca

Introduction

There are differing views regarding the potential benefits of mycorrhizal associations for tree–water relations. In many studies, mycorrhizal fungi have been shown to improve root water uptake and mitigate the effects of water-deficit stress (Davies et al. 1996; Morte et al. 2001; Landhäusser et al. 2002; Muhsin and Zwiazek 2002a, b; Marjanović et al. 2005), although other studies have shown little effect or reduced root hydraulic conductance by ectomycorrhizal (ECM) associations (Coleman et al. 1990; Nardini et al. 2000; Calvo Polanco et al. 2008; Yi et al. 2008). In *Populus tremula* × *tremuloides*, mycorrhiza formation resulted in an increased transcript level for the most prominently expressed PIP1 and PIP2 aquaporin genes in roots and increased root hydraulic conductivity (Marjanović et al. 2005). While mycorrhizal-induced expression of aquaporins may facilitate root water transport (Marjanović et al. 2005), some mycorrhizal associations have increased mortality of the host plants and resulted in leaf chlorosis (Cripps 2001; Calvo Polanco et al. 2008) and in reduced root volume (Nguyen et al. 2006).

The response of trees to mycorrhizae may be affected by environmental conditions (Johnson et al. 1997; Zhou and Sharik 1997), age, and nutritional status of host tree species at the time of inoculation (Corrêa et al. 2006), and specificity of plant–mycorrhizal associations (Molina et al. 1992a). The type of association developed between the plant and the fungus may also affect the extent of any measurable benefits to plants (Coleman et al. 1990). Most boreal forest trees form only ECM associations, although in some tree genera such as *Salicaceae* and *Ulmaceae*, vesicular–arbuscular mycorrhizal (VAM) associations can also form depending upon environmental and other conditions (Smith and Read 1997; Brundrett 2002). Addition-

ally, E-strain fungal species such as *Wilcoxina* spp. are known to form ectendomycorrhizal (EEM) associations with *Pinus* and *Larix* genera. Since E-strain fungi can form either EEM or ECM structures depending upon the tree species and because EEM fungi are thought to have a broad global distribution with a wide range of plant hosts (Molina et al. 1992a, b), it has been suggested that tree species outside of the *Pinus* and *Larix* genera may be capable of forming EEM associations (Peterson et al. 2004). Weakly pathogenic fungal associations were previously misinterpreted as EEM associations, largely indicated and characterized by the presence of intracellular hyphae, although no definitive evidence of EEM associations in deciduous tree species has been experimentally observed to date with current standards for defining true EEM associations (reviewed in Trevor et al. 2001).

Balsam poplar (*Populus balsamifera* L. subsp. *balsamifera*) is one of the dominant trees in the northern mixed wood forests in North America. The roots of balsam poplar are commonly associated with both VAM and ECM fungi (Helm and Carling 1993). In the present study, we determined that balsam poplar forms EEM associations with *W. mikolae* var. *mikolae* (Wm) and ECM associations with *Hebeloma crustuliniforme* (Hc). Subsequently, we studied the effects of these associations on growth and water relations of the host plants inoculated with these fungi.

Materials and methods

Mycorrhizal inocula

H. crustuliniforme (Bull.) Quél. (University of Alberta Microfungus Collection and Herbarium, UAMH 5247) and *W. mikolae* var. *mikolae* (Yang and Wilcox) Yang and Korf (University of Alberta Microfungus Collection and Herbarium, UAMH 6703) were sub-cultured on Melin–Norkrans media (MNM) agar (Mason 1980). The fungi were sub-cultured in aerated MNM liquid media on an orbital shaker and grown for 4 weeks under sterile conditions to produce liquid inoculum for seedling roots.

Plant material

For root microscopy, balsam poplar (*Populus balsamifera* L. subsp. *balsamifera*) seeds were locally collected from the North Saskatchewan River Valley, Edmonton, Alberta, Canada. The seeds were surface-sterilized in 5% sodium hypochlorite, thoroughly rinsed with deionized water, and germinated for 4 days in Petri dishes on sterile silica sand. Germinants were transferred to styroblock™ trays (superblock 160/60, Beaver Plastics, Edmonton, AB, Canada) containing washed, sterile silica sand, and grown in a controlled-environment growth chamber [60% RH, 18 h photoperiod, 22°C/18°C day/night, and 300 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) with full-spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, Ontario, Canada]. Trays were bottom-watered every second day with deionized water. After 4 weeks, randomly selected seedlings were removed from the trays, and roots were rinsed free of sand and aseptically transferred in a sterile laminar flow hood to autoclaved double Magenta™ tissue culture vessels (V8505, Sigma-Aldrich) attached with Magenta™ couplers (C0667, Sigma-Aldrich) containing washed, sterile, fine silica sand. During transplantation, seedling roots were surface-inoculated with 5 mL homogenized *H. crustuliniforme* (Hc) or *W. mikolae* var. *mikolae* (Wm) mycelia per seedling root using a wide-tip sterile pipette. A minimum of eight seedlings were transplanted but not inoculated to serve as non-inoculated controls. Magenta™ vessels containing seedlings were sealed and returned to the growth chamber. Four, 8, and 12 weeks following transplantation, 25 mL of 0.05% 20–20–20 (N–P–K) fertilizer was added to the containers. Magenta™ vessels were slightly vented periodically to ensure adequate aeration, with precautions taken to prevent possible contamination from air-borne fungi and bacteria. Seedlings were grown for a total of 16 weeks after germination prior to microscopic examination of roots.

For growth and physiological measurements, balsam poplar shoot cuttings were collected from 7- to 10-year-old balsam poplar trees (minimum of 20 trees sampled in Edmonton, Alberta, Canada) in late winter prior to bud-break. Cuttings from the previous year's growth, 10–15 cm in length with at least one dormant bud each, were wrapped in plastic foil and placed in dark storage at 4°C for up to 3 weeks. Prior to use, cuttings were moved to room temperature, rinsed with 75% ethanol solution for about 1 min, rinsed, and then soaked in sterile deionized water for 3 days. Cuttings were inserted into 12.5-cm diameter (1-L volume) cheesecloth-lined pots filled with sterile, washed coarse sand, which covered most of the cuttings up to the middle of the topmost bud. Pots were randomly arranged in a controlled-environment growth chamber (60% RH, 18 h photoperiod, 22°C/18°C day/night, 300 μmol m^{-2} s^{-1} PPFD with full-spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, Ontario, Canada). Most cuttings exhibited bud-break and leaf growth within 2 weeks following placement in the growth chamber and were top-watered every other day with deionized water. Cuttings that did not flush were removed from the experiment. After 4 weeks, cuttings were divided into four groups. One group was inoculated with Hc (n=8) by injecting 5 mL total of homogenized mycelia with a wide-

tip sterile pipette into the sand at about 3 cm below the surface and in two to three locations close to the stem. The second group of cuttings was inoculated with Wm (n=8) in the same manner. The remaining two groups served as non-inoculated controls for Hc (n=8) and Wm (n=8) plants. Control cuttings were inoculated with 5 mL autoclaved, fungal-free MNM liquid media in the same manner as for the mycorrhizal seedlings. Pots were grouped according to treatment by placing them into shallow, lined trays, and treatment groups were kept physically separated from each other to prevent cross-contamination by fungi between treatments. Plants were top-watered every second day with a small quantity of deionized water for 2 weeks following inoculation. During the third week following inoculation, plants were bottom-watered with deionized water every second day. The following week, cuttings were also bottom-watered with 0.05% 20–20–20 (N–P–K) fertilizer solution once per week in addition to their regular watering schedule. Plants were grown for 12 weeks following inoculation to ensure adequate colonization prior to measurements.

Root microscopy

Randomly selected seedlings were removed from Magenta™ vessels and gently rinsed free of sand. Several root segments that appeared to be mycorrhizal, based upon their darker root coloration and their shorter, thicker appearance compared to non-mycorrhizal roots (Brundrett et al. 1996), were selected for harvesting. Distal <0.5-cm-long root sections were excised with a razor, and fixed for 5 days in 2.5% glutaraldehyde in 0.1 M HEPES buffer, pH 6.8. The root segments were subsequently embedded in LR-White resin and sectioned for microscopy (Melville et al. 1998). Several cross-sections were made of the root sections with a glass-knife ultramicrotome along the lengths of the root segments and prepared for laser scanning confocal microscopy. Sections were stained for 90 s with 0.5% sulforhodamine G. Permount was used to preserve the sections.

Shoot growth, leaf area, leaf water potential, and stomatal conductance

Shoot height measurements were taken in intact plants, from the base of the stem to the shoot tip. Stem diameters were measured with calipers at the sand level. Projected total leaf surface area for each plant was determined with an LI-3100C leaf surface meter (LI-COR, Lincoln, NE, USA).

Shoot water potential (Ψ_w) measurements were conducted for the entire shoots as previously measured (Wan et al. 1999) using a Scholander pressure chamber.

Leaves from the mid-region of stems were used for stomatal conductance (g_s) measurements in intact cuttings within the growth chamber, using an LI-1600 steady-state porometer (LI-COR) at approximately 10 A.M. on the day of harvest.

Root hydraulic conductivity and root volume

To prevent the flow of water through the surface of the stem cutting during root pressurization, pressure-resistant silicone sealant was applied to the upper surface of the cuttings 3 days prior to harvesting. For the measurements of root hydraulic conductivity (L_{pr}), half of the plants from each treatment were detopped so that approximately 6–8 cm of the new shoot remained attached to the root system. It was assumed that axial root conductivity provided the greatest resistance of all pathways contributing to total root water flux (Steudle and Peterson 1998), therefore the slight differences in stem length were considered to have a negligible effect on L_{pr} measurements. Roots were placed in a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA) in deionized water, which was continuously aerated with a magnetic stir bar (Wan and Zwiazek 1999). Root water flow rate (Q_v, $m^3\ s^{-1}$) was measured for a minimum of 20 min at hydrostatic pressures of 0.3, 0.6, and 0.9 MPa, which are within a linear range of Q_v responses to pressure (Siemens and Zwiazek 2003, 2004). A minimum interval of 10 min was maintained between Q_v measurements at each increasing pressure to stabilize Q_v values. Once initial root water flux (J_v, $m^3\ s^{-1}\ MPa^{-1}$) measurements were made, values were divided by root volume to obtain root hydraulic conductivity (L_{pr}, $s^{-1}\ MPa^{-1}$). Root volume has been used previously in several publications as an acceptable unit of measurement for adjustment of J_v measurements (Landhäusser et al. 2002; Muhsin and Zwiazek 2002a, b; Voicu and Zwiazek 2004).

Root volumes were measured for each root system using volume displacement of water in a graduated cylinder (Voicu and Zwiazek 2004).

Activation energy and xylem exudate PTS_3 concentration

To detect changes in root water flow pathways and the contribution of aquaporins to root water flow, we have used the apoplastic tracer dye, and we have measured activation energy for root water flow. Activation energy of root water flux (E_a, kcal mol^{-1}) was measured using Scholander pressure chambers (Wan and Zwiazek 1999). Roots were immersed in an aqueous solution of 0.02% trisodium 3-hydroxy-5,8,10-pyrenetrisulfonic acid (PTS_3). Since this fluorescent tracer dye is not transported across cell membranes, this method has been used to measure relative changes in water transport pathways (Siemens and Zwiazek 2003; Schaider et al. 2006). A stirring plate was placed

underneath each pressure chamber, and the PTS_3 solution was continuously aerated with a magnetic stirring bar. A circulating water bath (Haake C, F3 digital control, Thermo Electron, W. Germany) connected to a hollow copper coil inserted into the root bathing solution was used to adjust the temperature of the solution inside the pressure chambers by cooling the copper coils immersed in the root bathing solution. A digital thermometer probe was inserted into one of the pressure chambers to monitor actual temperature changes of the root bathing solution. The measurements of Q_v were collected at constant 0.3 MPa hydrostatic pressure for a minimum of 20 min at each decreasing temperature (25°C, 20°C, 15°C, 10°C, and 5°C), with a minimum of 15 min interval between Q_v measurements at each temperature for stabilization of Q_v values. Activation energy was calculated from Arrhenius plots of the slope of $\ln(L_{pr})$ and temperature $(K)^{-1}$ using the formula:

$$\mathrm{Ea} = -\left[R \times \ln K_2 - \ln K_1\right) / \left(T_2^{-1} - T_1^{-1}\right)\right]$$

where

R 1.987×10^{-3} kcal mol^{-1} T^{-1}
K_2 L_{pr} at point 2
K_1 L_{pr} at point 1
T_2 temperature (K) at point 2
T_1 temperature (K) at point 1

Points 1 and 2 were selected from the linear regions of Arrhenius plots.

For measurement of xylem exudate PTS_3 concentrations, xylem sap samples were collected from pressurized roots following each set of Q_v measurements at each temperature. Samples were diluted with water and PTS_3 concentration measured against a PTS_3 standard curve using a Sequoia–Turner 450 spectrofluorometer (Apple Scientific, Chesterland, OH, USA) with a 405-nm excitation and 515-nm emission spectrum (Skinner and Radin 1994). PTS_3 concentrations for each root system were also plotted against temperature for each root system.

Root colonization

Whole root systems were gently washed and stored in 4:3:3 (by volume) ethanol–glycerol–water at 4°C prior to analysis. A sub-sample of roots from each treatment was selected for root colonization determinations. Root systems were removed from preserving solution and dissected to collect all fine roots with a diameter of <0.05 mm. These fine roots were cut into 1- to 2-cm length pieces, randomly mixed, and spread out in a large, water-filled Petri dish. Approximately one-quarter of the fine roots were removed from the batch and stored in sealed tubes filled with fresh preserving solution. Percent length root colonization determinations of mycorrhizal and non-mycorrhizal roots were conducted according to the procedure of Brundrett et al. (1996), with slight changes made to clearing and staining times that were required for balsam poplar roots. Sufficient clearing of roots with 10% KOH solution in an autoclave occurred within 20 min, which was followed by 12 min of root staining with 0.05% Trypan blue in an autoclave, and approximately 5 days of destaining in lactoglycerol, prior to mycorrhizal root segment counts in grid-lined Petri dishes.

Statistical analysis

Data were analyzed using a single-factor randomized block design mixed ANOVA with SAS 9.1. (SAS Institute, NC, USA) to statistically compare the two inoculation treatments (Hc and Wm) with each other and with the non-inoculated control (CTRL) group (α=0.05). All data were first tested for normality of distribution and homogeneity of variance using residuals, and any statistical outliers were removed from data sets.

Results

Comparison of shoot measurements between treatments

Stomatal conductance, shoot height, total leaf area, stem diameter, and shoot water potential

Both stomatal conductance (g_s; Fig. 1a) and shoot height (Fig. 1b) of plants inoculated with *W. mikolae* var. *mikolae* (Wm) plants were approximately 30% higher ($p<0.05$) compared to those inoculated with *H. crustuliniforme* (Hc) and non-inoculated control (CTRL). There were no significant differences in g_s and shoot height between the Hc and the CTRL plants (Fig. 1a,b). There was also a large increase in the total leaf area of Wm plants, and this increase was statistically significant compared with Hc plants (Fig. 1c).

Stem diameter increased in Hc and decreased in Wm plants relative to CTRL with significant ($p\leq0.05$) differences between Hc and CTRL plants (Fig. 2a).

There were no statistically significant differences in shoot Ψ_w between the three groups of plants (CTRL, Hc, and Wm; Fig. 2b).

Comparison of root measurements between treatments

Activation energy and changes in PTS_3 concentration with temperature

There were no statistically significant differences in activation energy of root water flux (E_a) or in changing PTS_3 concentration with decreasing temperature (ΔPTS_3) between the three groups of plants (CTRL, Hc, and Wm; Fig. 2c,d).

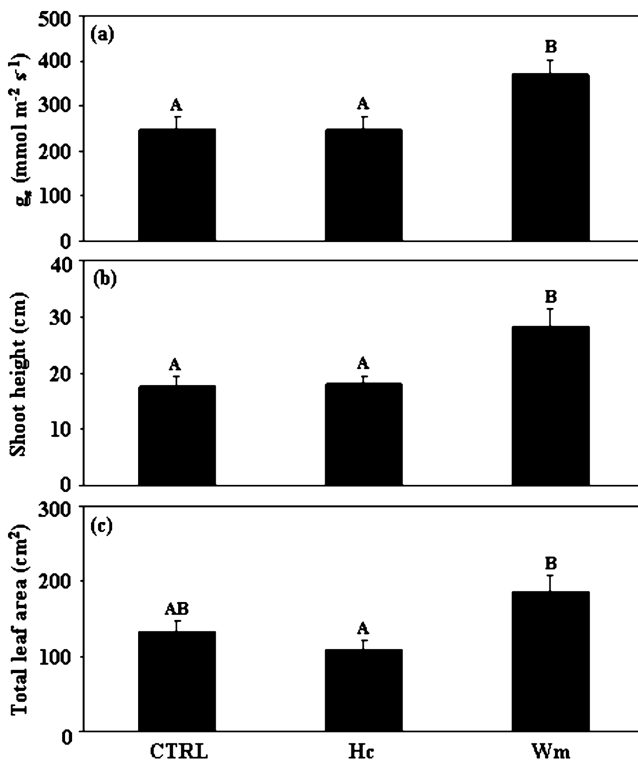

Fig. 1 **a** Stomatal conductance (g_s), **b** shoot height, and **c** total leaf area in non-mycorrhizal (*CTRL*) or mycorrhizal balsam poplar seedlings inoculated with *Hebeloma crustuliniforme* (*Hc*) or *Wilcoxina mikolae* var. *mikolae* (*Wm*). Least-squares means±SE are shown (minimum, n=8). Significant (p<0.05) differences between treatments are indicated by *uppercase letters*

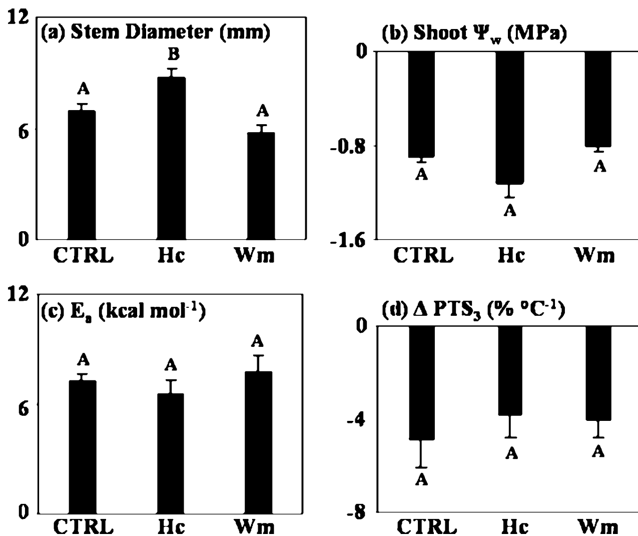

Fig. 2 **a** Stem diameter, **b** shoot water potential (Ψ_w), **c** activation energy (E_a), and (**d**) changes in PTS_3 xylem exudate concentration with decreasing temperature (ΔPTS_3) in non-mycorrhizal (*CTRL*) or mycorrhizal balsam poplar seedlings inoculated with *Hebeloma crustuliniforme* (*Hc*) or *Wilcoxina mikolae* var. *mikolae* (*Wm*). Least-squares means±SE are shown (minimum, n=8). Significant (p<0.05) differences between treatments are indicated by *uppercase letters*

Root colonization and root hydraulic conductivity

Both Hc and Wm plants had approximately a twofold higher (p<0.05) percentage of colonized root length compared with CTRL plants (22.34%±2.89%), with no significant differences in colonization percentages between Hc and Wm plants (Fig. 3a).

Root volume was significantly higher in Wm but not Hc plants compared with non-inoculated control (Fig. 3b). Root volume in Wm plants was also significantly higher than that in Hc plants (Fig. 3b). In contrast, L_{pr} was 1.5-fold higher in Hc compared with non-inoculated plants ($3.19\times10^{-5}\pm0.49\times10^{-6}$ in Hc and $2.00\times10^{-5}\pm3.35\times10^{-6}$ s^{-1} MPa^{-1} in CTRL; Fig. 3c). The difference in L_{pr} between the Wm and the CTRL plants was not statistically significant (Fig. 3c).

Comparative anatomy of colonized roots

Several images of embedded and stained balsam poplar root segments colonized with either Hc or Wm were taken and analyzed, with the clearest and most representative images presented in Fig. 4. Laser scanning confocal microscopy

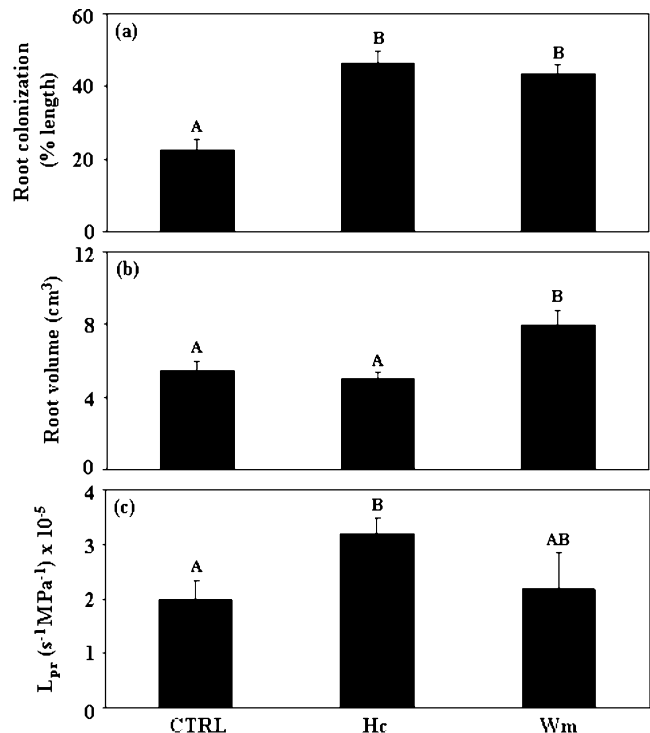

Fig. 3 **a** Root colonization, **b** root volume, and **c** root hydraulic conductivity (L_{pr}) in non-mycorrhizal (*CTRL*) or mycorrhizal balsam poplar seedlings inoculated with *Hebeloma crustuliniforme* (*Hc*) or *Wilcoxina mikolae* var. *mikolae* (*Wm*). Least-squares means±SE are shown (minimum, n=8). Significant (p<0.05) differences between treatments are indicated by *uppercase letters*

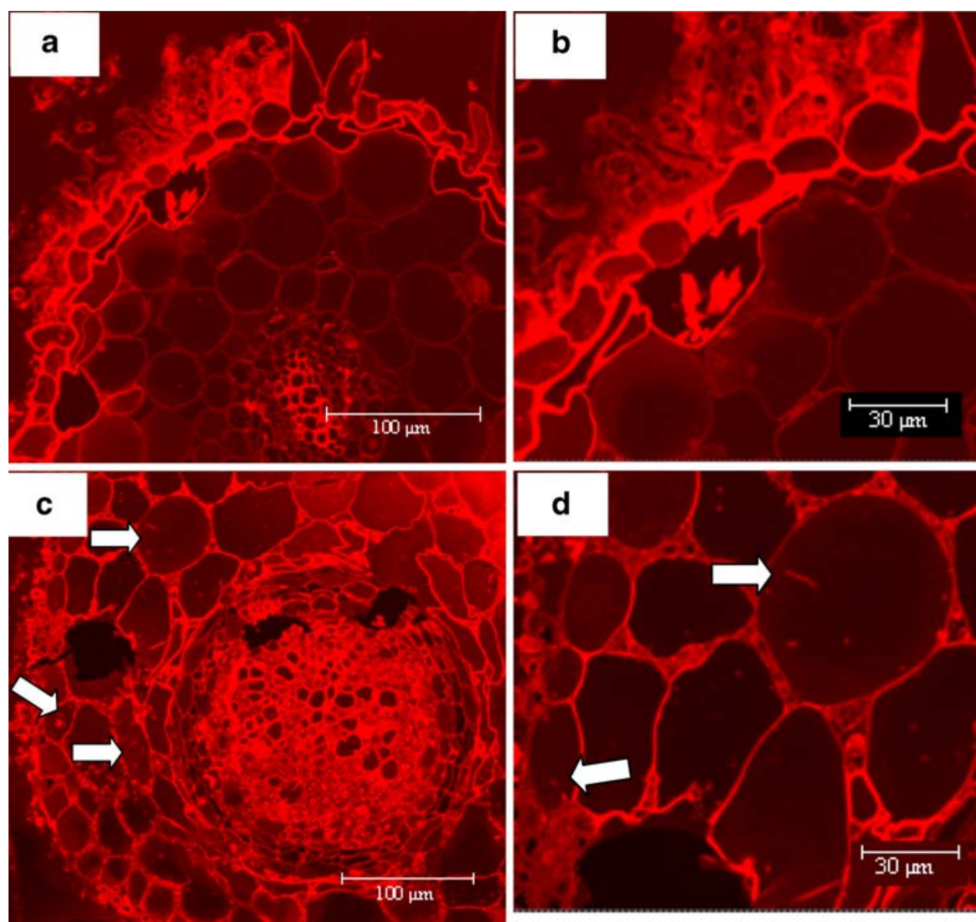

Fig. 4 Images of 4-month-old balsam poplar seedling roots inoculated with *Hebeloma crustuliniforme* and *Wilcoxina mikolae* var. *mikolae*. Cross-sectional (**a**) and magnified (**b**) images of roots inoculated with *H. crustuliniforme*, and cross-sectional (**c**) and magnified (**d**) images of roots inoculated with *W. mikolae* var. *mikolae* roots are shown. Root segments were embedded in LR-White, sectioned at least 5 mm from the root tip, and stained with sulforhodamine G for anatomical examination with laser scanning confocal microscopy. *Arrows* indicate fluorescing intracellular structures consistent with intracellular hyphae

images of balsam poplar roots inoculated with Hc and Wm, and stained with sulforhodamine G show different distributions of hyphae (Fig. 4). In the Hc root sections, a mantle layer, indicative of an ectomycorrhizal association, is clearly visible around the outer part of the root, but no distinct Hartig net is visible (Fig. 4a,b). In the Wm root, a mantle surrounding the outside of the root and an extensive Hartig net that is located between cortical cells and extending to the vascular cylinder are present (Fig. 4c,d). The fluorescing structures (indicated by arrows) that are present inside cortical cells in Wm roots) are consistent with intracellular hyphae indicative of an ectendomycorrhizal association (Fig. 4d).

Discussion

Two separate studies are presented in this paper. The first was to determine if Hc and Wm were capable of forming ECM and EEM associations with balsam poplar seedling roots, respectively. The second was to determine what effect these two fungal species had on rot water relations of balsam poplar cuttings. It is possible that the results and conclusions drawn from these two individual studies may differ somewhat, but it was assumed that the anatomical nature of the mycorrhizal–plant root associations would be consistently similar between the two studies.

In the microscopy study, it was assumed that many of the observed fluorescing structures other than the plant cell walls were mycorrhizal hyphae, as per the microscopy procedure of Melville et al. (1998), which recommended the use of the sulforhodamine G dye to observe mycorrhizal structures in root sections. Hc formed an ECM association with balsam poplar, as indicated by a partial mantle with hyphal projections around sectioned roots. However, there was little evidence of a Hartig net around the first layer of cortical cells (Fig. 4a,b). The fact that Hc did not appear to form a full mantle and Hartig net was surprising since the fungus has been reported to form ECM associations with a broad host range (Smith and Read 1997) including *Populus* (Hutchison 1991; Yi et al. 2008). Wm appeared to form characteristic EEM structures (Trevor et al. 2001; Peterson et al. 2004) consisting of a full mantle and a Hartig net that extended through the root cortex (Fig. 4c,d). We also observed fluorescing structures inside the cortical cells of Wm-colonized roots

that were tentatively identified as intracellular hyphae. These hyphae usually occur in senescing roots of plants forming EEM associations and may not be present in all mycorrhizal roots (Trevor et al. 2001; Peterson et al. 2004). Wm is an E-strain fungus capable of forming ECM and EEM structures (Peterson et al. 2004). To the best of our knowledge, this is the first report of an EEM association between Wm and angiosperm tree species. Since this association was produced in a controlled growth chamber environment, further studies will be needed to determine whether they also form in balsam poplar trees growing in their natural environment.

In contrast to ECM associations, little is known about physiological responses of trees to EEM colonization. Wm resulted in significantly greater increases in the leaf area, stomatal conductance (g_s), shoot height (Fig. 1), and root volume (Fig. 3b) but had no significant effect on shoot water potential (Ψ_w; Fig. 2) compared to Hc, which appeared to have no effect upon these parameters. In different studies, ECM fungi increased (Muhsin and Zwiazek 2002a; Nguyen et al. 2006), decreased (Corrêa et al. 2006; Nguyen et al. 2006), or had no effect (Landhäusser et al. 2002; Corrêa et al. 2006) on plant growth. The effect of ECM fungus may depend on the age, stage of development, and nutritional status of plants (Corrêa et al. 2006).

In contrast to growth responses, Hc resulted in significantly higher root hydraulic conductivity (L_{pr}) than the CTRL plants (Fig. 3c) compared with the difference between the CTRL and the Wm plants (not significant). In previous studies, Hc increased L_{pr} in several studied tree species including *Picea mariana* (Landhäusser et al. 2002; Muhsin and Zwiazek 2002b), *Ulmus americana* (Muhsin and Zwiazek 2002a), and *Populus tremuloides* (Landhäusser et al. 2002). A similar increase was also reported for the *Populus tremula* × *tremuloides* association with *Amanita muscaria* (Marjanović et al. 2005). However, Hc has also been reported to have no effect on L_{pr} in *Betula papyrifera*, *Populus tremuloides* (Yi et al. 2008), and *U. americana* (Calvo Polanco et al. 2008). Similarly, *Tuber melanosporum* had no effect on L_{pr} of *Quercus ilex* (Nardini et al. 2000). The increase in L_{pr} of ECM roots may be due to both apoplastic and aquaporin-mediated root water transport (Muhsin and Zwiazek 2002a; Marjanović et al. 2005). The proportion of apoplastic vs. cell-to-cell root water flux may, in turn, be affected by numerous environmental and internal factors, including drought (Taleisnik et al. 1999; Siemens and Zwiazek 2004), salinity, and osmotic stress (Carvajal et al. 1999; Engels 1999), and anatomical changes in root structure (Hose et al. 2001). The present study demonstrated that hyphal penetration of the root cortex by Wm had little effect on root hydraulic properties in contrast to Hc, which only penetrated the outer root layers. These results suggest that L_{pr} in mycorrhizal plants may be largely due to specific biochemical changes triggered by the fungus in the root tissue.

In the present study, Wm and Hc plants showed slight but non-significant changes in both E_a and PTS_3 concentrations in xylem exudate (Fig. 2), which may have contributed to but could not fully explain the differences in L_{pr} between the two mycorrhizal treatments. E_a, a measure of energy required for water transport across cell membranes, varied between 6.55–7.8 kcal mol^{-1}, with a lower value for Hc than for the CTRL or Wm plants. Previous cell membrane transport studies reported that $E_a <$ 6 kcal mol^{-1} indicates aquaporin (AQP)-mediated passive water transport through individual membranes, with increased E_a values due to restriction of AQP-mediated transport (Maurel 1997; Shütz and Tyerman 1997). However, such a threshold E_a value may not be applicable to whole-plant tissues or root systems, which have multiple resistance pathways. If non-AQP-mediated transport (i.e., apoplast) becomes a greater contributor to overall L_{pr} than cell-to-cell transport, overall E_a values may decrease due to less resistance provided by the apoplastic pathway. In the present study, Hc plants had a slightly lower E_a value, which may partially explain higher L_{pr} values. However, the large differences in L_{pr} seen between the mycorrhizal and the CTRL plants (Fig. 3c) cannot be entirely explained by the non-significant changes in either the relative apoplastic flux (estimated by PTS_3 concentration) or the resistance to root water flux (estimated by E_a).

The cohesion–tension (CT) theory of water uptake describes the upward movement of water through the xylem as a tightly controlled cause-and-effect relationship between g_s, Ψ_w, and hydraulic conductivity (Tyree 1997). However, the differences in L_{pr} between Hc and Wm in the present study cannot be explained by the differences in g_s. The overall rate of water flux is determined by the largest resistance to water flux within the whole-plant system. Any change in one of these factors could result in predicted changes in the other factors, based upon the principles of the CT theory and the soil–plant–atmosphere continuum (Sperry et al. 2003). The lack of a correlation between these parameters in both Hc and Wm may be indicative of both the fact that pressure chamber measurements of L_{pr} in this study were representative of the potential maximum L_{pr} that can be reached in the absence of other limiting factors within the whole-plant system and that mycorrhizal-induced changes in plants may alter physiological plant processes such as those involved in plant water relations. Alternatively, mycorrhizal associations may have affected the largest resistance to water flux within balsam poplar. These results are consistent with a previous study, where increased L_{pr} in mycorrhizal roots was observed in white spruce and trembling aspen without a corresponding increase in g_s (Landhäusser et al. 2002).

In the present study, Wm increased shoot growth in balsam poplar without measurably affecting whole-root water relations, and the opposite was observed for Hc, suggesting that underlying biochemical and molecular mechanisms may be responsible for the differences observed in the effects of the two fungal species. Symbiosis-associated changes at the cell level, such as differential expression of genes and transport proteins (Lei and Dexheimer 1988; Duplessis et al. 2002; Tagu et al. 2002; Marjanović et al. 2005) have been reported. However, a possible role of extrametrical hyphae in these responses cannot be excluded.

The different effects of Hc and Wm on growth and root hydraulic properties of balsam poplar may be partly due to the type of association formed by Hc (ECM) and Wm (EEM), as well as differences between the two fungal species (Coleman et al. 1990). Root length colonization by Hc and Wm was similar (~50%). Because plants inoculated with different mycorrhizal species were isolated from each other, it was assumed that the measured colonization percentages were indicative of the extent of root colonization by that specific mycorrhizal species. However, it is acknowledged that the method used in this study (Brundrett et al. 1996) measures total colonization without determination of individual fungal species. Therefore, differences in the observed growth and physiological responses between Hc and Wm treatments were likely due to the effects of the mycorrhizal treatments rather than the extent of root colonization. Metabolic, developmental, and genetic changes associated with hyphal penetration of roots have been well documented in both plant hosts and fungi at the start of the ECM symbiosis (Smith and Read 1997; Frettinger et al. 2007). However, such changes have not been well studied with respect to EEM associations. Little is also currently known about the EEM root–hyphal interface, its properties, and its effects on host plants, although it is thought that EEM fungi may provide some benefit to *Pinus* and *Larix* host trees (Peterson et al. 2004). Due to the nature of Wm as an E-strain fungus, it is possible that intracellular hyphal penetration of balsam poplar roots by Wm was partly responsible for the observed differences in the effects of the two fungal species. It is possible that, compared to root transfer cells that facilitate substance transfer between the fungal hyphae and root cells in some ECM fungal associations (Ashford and Allaway 1985; Cairney et al. 1994), intracellular hyphae may provide a more direct conduit to the transfer of intracellular substances, soil nutrients, and water between fungus and plant symbionts. However, more research is needed to understand the dynamics and effects of both ECM and, in particular, EEM associations. Additionally, further research is required to better understand the ecological role fulfilled by EEM fungi, particularly in association with angiosperm tree species, and how the biochemical and cellular processes that occur during ECM and EEM symbiosis may affect the host plant.

Acknowledgment The authors thank Dr. R. L. Peterson (University of Guelph) for assistance with interpretation of microscopic images, Dr. R. Currah (University of Alberta) for advice with culturing and growing mycorrhizal seedlings, and Dr. M. D. Jones (Okanagan University) for advice with root colonization measurements. The authors also acknowledge the Biological Microscopy Services Unit (University of Alberta) for their assistance with the microscopy portion of this study. This work was supported in part by the Natural Sciences and Engineering Research Council of Canada (NSERC) in the form of a postgraduate scholarship to J.A.S. and a Discovery Research Grant to J.J.Z.

References

Ashford AE, Allaway WG (1985) Transfer cells and Hartig net in the root epidermis of the sheathing mycorrhizal of *Pisonia grandis* R.Br. from Seychelles. New Phytol 100:595–612 doi:10.1111/j.1469-8137.1985.tb02806.x

Brundrett M (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154:275–304 doi:10.1046/j.1469-8137.2002.00397.x

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture, Australian Centre for International Agricultural Research. CSIRO Publishing, Victoria, Australia

Cairney JWG, Rees BJ, Allaway WG, Ashford AE (1994) A basidiomycete isolated from *Pisonia* mycorrhizal forms sheathing mycorrhizas with transfer cells on *Pisonia grandis* R.Br. N Phytol 126:91–98 doi:10.1111/j.1469-8137.1994.tb07533.x

Calvo Polanco M, Zwiazek JJ, Voicu MC (2008) Responses of ectomycorrhizal American elm (*Ulmus americana*) seedlings to salinity and soil compaction. Plant Soil 308:189–200 doi:10.1007/s11104-008-9619-z

Carvajal M, Martínez V, Alcaraz CF (1999) Physiological function of water channels as affected by salinity in roots of paprika pepper. Physiol Plant 105:95–101 doi:10.1034/j.1399-3054.1999.105115.x

Coleman MD, Bledsoe CS, Smit BA (1990) Root hydraulic conductivity and xylem sap levels of zeatin riboside and abscisic acid in ectomycorrhizal Douglas fir seedlings. New Phytol 115:275–284 doi:10.1111/j.1469-8137.1990.tb00453.x

Corrêa A, Strasser RJ, Martins-Loução MA (2006) Are mycorrhiza always beneficial? Plant Soil 279:65–73 doi:10.1007/s11104-005-7460-1

Cripps CL (2001) Mycorrhizal fungi of aspen forests: natural occurrence and potential applications. USDA Forest Service Proceedings RMRS-P-18.

Davies FT Jr, Svenson SE, Cole JC, Phavaphutanon L, Duray SA, Olalde-Portugal V et al (1996) Non-nutritional stress acclimation of mycorrhizal woody plants exposed to drought. Tree Physiol 16:985–993

Duplessis S, Tagu D, Martin F (2002) Living together in the underground a molecular glimpse of the ectomycorrhizal symbiosis. In: Osiewacz HD (ed) Molecular development of fungal development. Marcel Dekker, New York, USA, pp 297–323

Engels C (1999) Regulation of xylem transport of calcium from roots to shoots of maize by growth-related demand. J Plant Nutr Soil Sci 162:287–294 doi:10.1002/(SICI)1522-2624(199906)162:3<287::AID-JPLN287>3.0.CO;2-N

Frettinger P, Derory J, Herrmann S, Plomion C, Lapeyrie F, Oelmüller R, Martin F, Buscot F, (2007) Transcriptional changes in two types of pre-mycorrhizal roots and in ectomycorrhizas of oak microcuttings inoculated with *Piloderma croceum*. Planta 225:331–340 doi:10.1007/s00425-006-0355-4

Helm DJ, Carling DE (1993) Use of soil transfer for reforestation on abandoned mined lands in Alaska I. Effects of soil transfer and phosphorus on growth and mycorrhizal formation by *Populus balsamifera*. Mycorrhiza 3:97–106

Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W (2001) The exodermis: a variable apoplastic barrier. J Exp Bot 52:2245–2264 doi:10.1093/jexbot/52.365.2245

Hutchison LJ (1991) Description and identification of cultures of ectomycorrhizal fungi found in North America. Mycotaxon 42:387–504

Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol 135:575–586 doi:10.1046/j.1469-8137.1997.00729.x

Landhäusser SM, Muhsin TM, Zwiazek J (2002) The effect of ectomycorrhizae on water relations in aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) at low soil temperatures. Can J Bot 80:684–689 doi:10.1139/b02-047

Lei J, Dexheimer J (1988) Ultrastructural localization of ATPase activity in the *Pinus sylvestris*/*Laccaria laccata* ectomycorrhizal association. New Phytol 108:329–334 doi:10.1111/j.1469-8137.1988.tb04170.x

Marjanović Ž, Uehlein N, Kaldenhoff R, Zwiazek JJ, Wieß M, Hammp R et al (2005) Aquaporins in poplar: what a difference a symbiont makes!. Planta 222:258–268 doi:10.1007/s00425-005-1539-z

Mason PA (1980) Aseptic synthesis of sheathing (ecto-) mycorrhizas. In: Ingram DS, Helgeson JP (eds) Tissue culture methods for plant pathologists. Blackwell, Oxford, UK, pp 173–178

Maurel C (1997) Aquaporins and water permeability of plant membranes. Annu Rev Plant Physiol Plant Mol Biol 48:399–429 doi:10.1146/annurev.arplant.48.1.399

Melville L, Dickson S, Farquhar ML, Smith SE, Peterson RL (1998) Visualization of mycorrhizal fungal structures in resin embedded tissues with xanthene dyes using laser scanning confocal microscopy. Can J Bot 76:174–178 doi:10.1139/cjb-76-1-174

Molina R, Massicotte HB, Trappe JM (1992a) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF (ed) Mycorrhizal functioning—an integrative plant-fungal process. Chapman and Hall, New York, USA, pp 357–423

Molina R, Massicotte HB, Trappe JM (1992b) Ecological role of specificity phenomena in ectomycorrhizal plant communities: potential for interplant linkages and guild development. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB International, Wallingford, UK, pp 106–112

Morte A, Diaz G, Rodriguez P, Alarcon JJ, Sanchez-Blanco MJ (2001) Growth and water relations in mycorrhizal and non-mycorrhizal *Pinus halepensis* plants in response to drought. Biol Plant 44:263–267 doi:10.1023/A:1010207610974

Muhsin TM, Zwiazek JJ (2002a) Ectomycorhizas increase apoplastic water transport and root hydraulic conductivity in *Ulmus Americana* seedlings. New Phytol 153:153–158 doi:10.1046/j.0028-646X.2001.00297.x

Muhsin TM, Zwiazek JJ (2002b) Colonization with *Hebeloma crustuliniforme* increases water conductance and limits shoot sodium uptake in white spruce (*Picea glauca*) seedlings. Plant Soil 238:217–225 doi:10.1023/A:1014435407735

Nardini A, Salleo S, Tyree MT, Vertovec M (2000) Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. on hydraulic conductance and water relations of *Quercus ilex* L. seedlings. Ann Sci 57:305–312 doi:10.1051/forest:2000121

Nguyen H, Calvo Polanco M, Zwiazek JJ (2006) Gas exchange and growth responses of ectomycorrhizal *Picea mariana, Picea glauca*, and *Pinus banksiana* seedlings to NaCl and Na_2SO_4. Plant Biol 8:646–652 doi:10.1055/s-2006-924106

Peterson RL, Massicotte HB, Melville LH (2004) Mycorrhizas: anatomy and cell biology. NRC University Press, Ottawa, Canada

Schaider LA, Partker DR, Sedlak DL (2006) Uptake of EDTA-complexed Pb, Cd and Fe by solution- and sand-cultured *Brassica juncea*. Plant Soil 286:377–391 doi:10.1007/s11104-006-9049-8

Shütz K, Tyerman SD (1997) Water channels in *Chara corallina*. J Exp Bot 48:1511–1518

Siemens JA, Zwiazek JJ (2003) Effects of water deficit stress and recovery on the root water relations of trembling aspen (*Populus tremuloides*) seedlings. Plant Sci 165:113–120 doi:10.1016/S0168-9452(03)00149-3

Siemens JA, Zwiazek JJ (2004) Changes in root water flow properties of solution culture-grown trembling aspen (*Populus tremuloides*) seedlings under different intensities of water-deficit stress. Physiol Plant 121:44–49 doi:10.1111/j.0031-9317.2004.00291.x

Skinner RH, Radin JW (1994) The effect of phosphorus nutrition on water flow through the apoplastic by-pass of cotton roots. J Exp Bot 45:423–428 doi:10.1093/jxb/45.4.423

Smith SE, Read DJ (1997) *Mycorrhizal symbiosis*, 2nd edn. Academic, London, UK

Sperry JS, Stiller V, Hacke UG (2003) Xylem hydraulics and the soil–plant–atmosphere continuum: opportunities and unresolved issues. Agron J 95:1362–1370

Steudle E, Peterson CA (1998) How does water get through roots? J Exp Bot 49:775–788 doi:10.1093/jexbot/49.322.775

Tagu D, Lapeyrie F, Martin F (2002) The ectomycorrhizal symbiosis: genetics and development. Plant Soil 244:97–105 doi:10.1023/A:1020235916345

Taleisnik E, Peyrano G, Cordoba A, Arias C (1999) Water retention capacity in root segments differing in the degree of exodermis development. Ann Bot (Lond) 83:19–27 doi:10.1006/anbo.1998.0781

Trevor E, Yu JC, Egger KN (2001) Ectendomycorrhizal associations—characteristics and functions. Mycorrhiza 11:167–177 doi:10.1007/s005720100110

Tyree MT (1997) The cohesion–tension theory of sap ascent: current controversies. J Exp Bot 48:1753–1765

Voicu MC, Zwiazek JJ (2004) Cyclohexamide inhibits root water flow and stomatal conductance in aspen (*Populus tremuloides*) seedlings. Plant Cell Environ 27:199–208 doi:10.1111/j.1365-3040.2003.01135.x

Wan XC, Zwiazek JJ (1999) Mercuric chloride effects on root water transport in aspen (*Populus tremuloides*) seedlings. Plant Physiol 121:939–946 doi:10.1104/pp.121.3.939

Wan XC, Landhäusser SM, Zwiazek JJ, Lieffers VJ (1999) Root water flow and growth of aspen (*Populus tremuloides*) at low root temperatures. Tree Physiol 19:879–884

Yi H, Calvo Polanco M, MacKinnon MD, Zwiazek JJ (2008) Responses of ectomycorrhizal *Populus tremuloides* and *Betula papyrifera* seedlings to salinity. Environ Exp Bot 62:357–363 doi:10.1016/j.envexpbot.2007.10.008

Zhou M, Sharik TL (1997) Ectomycorhizal associations of northern red oak (*Quercus rubra*) seedlings along and environmental gradient. Can J Res 27:1705–1713 doi:10.1139/cjfr-27-11-1705