EFFECTS OF NANOSILVER-IMPREGNATION AND ALFALFA-INTERCROPPING ON FLUID TRANSFER IN DOWNY BLACK POPLAR WOOD

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ABSTRACT

Effects of alfalfa-intercropping and nanosilver-impregnation were studied here on the specific gas permeability of 10-year old *Populus nigra* var. *betulifolia*. Specimens were impregnated with a 200 ppm aqueous dispersion of silver nanoparticles under 250 kPa pressure; the results were then compared with the un-impregnated specimens. The size range of silver nanoparticles was 10 to 80 nm. After the initial measurement, specimens were kept in vaporized chamber for six months ($43\pm2°C$, and $80\pm3\%$ relative humidity); permeability was measured every month. Results showed that maximum specific longitudinal gas permeability was observed in the nanosilver-impregnated inner-wood specimens of the treatment with alfalfa ($1614,4 \times 10^{-13}$ m^3 m^{-1}), and minimum permeability was found in the un-impregnated outer-wood specimens of the treatment without alfalfa ($491,7 \times 10^{-13}$ m^3 m^{-1}) before the vaporization. Vaporized chamber made permeability decrease permeability at the first phases because of the absorption of water vapors and the consequent swelling; however, due to the proliferation of pits and perforation plates by fungi and molds, permeability was eventually increased. Antibacterial property of silver did not significantly decrease the growth of molds and fungi in the nanosilver-impregnated specimens.

Keywords: Alfalfa, Specific Gas Permeability, Diameter Increment, Intercropping, *Populus nigra betulifolia,* Vaporized Chamber.

INTRODUCTION

Poplar, as a fast-growing species, can satisfy the growing needs for solid woods; furthermore, it offers a quality raw material for wood-composite manufacturing factories because the end product will benefit from higher compression ratio. Poplar belongs to *Salicaceae* family. Apart from natural species, special capabilities of poplar hybrids and clones can be useful too. These improved trees grown on intensively managed plantations reach saw-timber size and are harvested at a younger age (Dickmann 2006). In the recent decades, intercropping of poplar trees with different crops was gradually increased (Chaudhry 2003). Such an agroforestry system is suitable to encourage farmers to produce wood along with their annual crop harvests. Alfalfa, a legume that fixes nitrogen in soil, is one of the best crops for agroforestry because it does not need annual plowing and sowing. The nitrogen fixing capacity of alfalfa is about 230 kg per hectare yearly (Taghiyari *et al.* 2011a). Use of nitrogen-fixing plants is one of the methods for fertilizing plantation trees (Zobel 1992). It was proved that nitrogen-fixing plants and nitrogen fertilizers have increasing effect on growth rate (DeBell *et al.* 2002a, DeBell *et al.* 2002b, Saarsalmi *et al.* 2006). In turn, any change in the growth pattern and growth rate of a tree may result in variation of wood properties (Zobel 1992). Permeability, as a physical property, in solid woods has significant impact on its applications and utilization in different industries (Taghiyari *et al.* 2012c). Many industries need to know how permeable their wood is so that they could use permeability values in decision-making processes for impregnation, drying, filtration, as well as other purposes.

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In hardwoods, longitudinal permeability value is more important than transversal (radial and tangential) values because of vessel elements orientation along longitudinal axis of trees. As to the fact that permeability is itself influenced by porosity and capillary structure of wood (Taghiyari 2013), the present study is, therefore, aimed at finding the effects of alfalfa-intercropping on diameter increment, as well as relationships between gas permeability and vessel properties of two treatments of with and without alfalfa-intercropping. A better knowledge on the effects, as well as final diameter increment and physical properties of the wood produced, may guide to taking a better agroforestry policy and managing the forest more efficiently. Furthermore, previous studies did not consider the possible difference between permeability of the inner and outer parts of the stem; so, findings in this regard would be quite applicable for the preservation process of poles and beams.

In the meantime, nanotechnology was used in many sciences, such as fluid transfer in porous media (Dashti *et al.* 2012, Ghorbani *et al.* 2012), enhancement of antibacterial and antimicrobial effects of silver (Narashimha *et al.* 2011), thermal conductivity (Kang *et al.* 2006), heat treatment (Taghiyari 2011), filtration, wood densification (Rassam *et al.* 2012), shape controlling (Sadeghi and Rastgo 2012), DNA detection (Ayesh and Awwad 2012), electrodeposition, semiconductors and molecular electronics (Soltaninezhad and Aminifar 2011, Pati 2012), hydrothermal synthesis (Alemi *et al.* 2012), missing data (Schmidt and Chen 2012), and many others. Furthermore, mineral and metal nanoparticles such as silver and wollastonite can both facilitate the heat-transfer (Taghiyari *et al.* 2012a, Taghiyari et al. 2012b, Taghiyari *et al.* 2012c) to the inner parts of the specimens, reduce hot press time (Taghiyari and Farajpour 2013, Taghiyari *et al.* 2013), improve fire-resistance (Rangavar *et al.* 2012, Taghiyari 2012b, Haghighi *et al.* 2013), and increase biological resistance of wood toward wood-deteriorating fungi and wood-insects, at the same time (Yu *et al.* 2012). Therefore its possible effects on the permeability of poplar specimens kept in vaporized chamber would be also beneficial to the wood impregnation and preservation industry.

MATERIALS and METHODS

Specimen Procurement
The present study was conducted on trees grown in a trial set up in the winter of 1999 with one year old rooted cuttings of *Populus nigra* var. *betulifolia* in an ongoing research project at The Iranian Research Institute of Forests and Rangelands. The trees were cut in the summer, 2008. Treatments included two sets of the poplar cuttings, both with the same initial spacing at 3 × 4 m; the first set was intercropped with alfalfa as N-fixing plant, and the second was without alfalfa. Three trees were selected from each treatment in such a way that their diameters at breast height were close to the mean diameter of their respected treatments. It is to be noted that due to some governmental regulations, more than three trees was not allowed to be cut; so, it was decided to select the trees close the mean diameter, so that the final results present a general mean value of the permeability properties of the trees. The planting of alfalfa was only continued for the first five years after the initial plantation of trees (that is, year 1 to year 5). For the last five years (year 6 to year 10), planting of alfalfa was discontinued. The reason why alfalfa was not planted for the last five years was due to the spread of poplar canopies that did not let enough light for the growth of the intercropped alfalfa. The alfalfa crops were harvested several times every year without plowing and sowing. To cut the permeability specimens, disks were first taken at breast height (1.3 m above the ground level) and 4 meter-height; they were then air-dried for 5 months.

Gas Permeability Specimen
Many techniques have been brought up to measure permeability in solid woods and wood-composite materials (Dermoe *et al.* 2012). In the present study, longitudinal gas permeability measurement apparatus was invented by the author based on the microstructure porosity of wood (Taghiyari 2012a) (Figure 1). Longitudinal specific gas permeability was determined using the falling-water volume-displacement

method (Shi 2007). Wood specimens were taken at random from the inner-wood and outer-wood areas. In order to eliminate the influence of moisture content on permeability, the wood samples were conditioned in a conditioning chamber at 23°C and relative humidity of 48±2% for four weeks; the moisture content of specimens was 10% when permeability tests were carried out. A round sample with known length as well as known cross-section area in m^2 is prepared to be placed in the holder of the apparatus and be tightly tide up with silicon hose and round hose clamps. The electronic time measurement device measures the time with milli-second precision. Once the time is measured, permeability values would be determined using following equations 1 and 2. Different diameter of glass tube can be easily used in the apparatus.
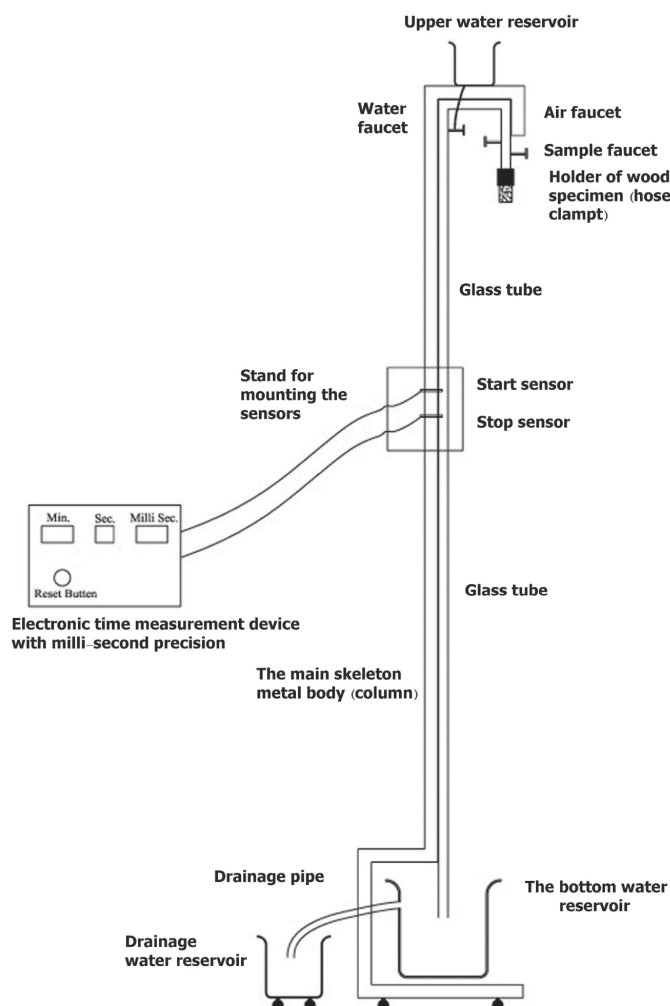

Figure 1. A schematic drawing of the gas permeability apparatus (USPTO No. US 8,079,249, B2) equipped with single-storey milli-second precision electronic time measurement device
(Taghiyari 2011b).

All gas permeability specimens were cylindrical in the present study, 18 mm in diameter and 30 mm long. Specimens were visually examined to be free from any knots, checks, and splits. The specimens were kept in conditioning chamber (23°C and relative humidity of 48±2%) to reach MC level of 10%. Both ends of each specimen were trimmed using sharp cutter blades. Furthermore, all around both longitudinal and radial specimens were covered by silicon adhesive to prevent air flow through radial and tangential directions. 20 specimens were cut randomly at scattered locations from each disk; 10

specimens at the first five year of growth (the inner-wood specimens), and 10 specimens at the last five year of growth (the outer-wood specimens). Furthermore, 10 specimens at radial direction were also cut. Measurements were carried out using falling water displacement volume method instructions (Taghiyari and Farajpour 2013). Connection between the specimen and holder of the apparatus was made fully air-tight. A pressure gauge with milli-bar precision was connected to the whole structure to monitor pressure gradient (ΔP) and vacuum pressure at any particular time as well as height of water column. Vacuum pressures at the starting and stopping points were 112 and 101,5 (minus milli-bar), respectively.

Three measurements were taken for each specimen; the average time was used for permeability calculation. Superficial permeability coefficient was then calculated using equations 1 and 2. The superficial permeability coefficients were then multiplied by the viscosity of air (μ=1,81×10^{-5} Pa s) for the calculation of the specific permeability ($K=k_g\,\mu$).

$$k_g = \frac{V_d CL(P_{atm} - 0.074\bar{z})}{tA(0.074\bar{z})(P_{atm} - 0.037\bar{z})} \times \frac{0.760mHg}{1.013 \times 10^6 \, Pa} \tag{1}$$

$$C = 1 + \frac{V_r(0.074\Delta z)}{V_d(P_{atm} - 0.074\bar{z})} \tag{2}$$

Where:

k_g	=	longitudinal specific permeability (m^3 m^{-1})
V_d	=	$\pi\, r^2 \Delta z$ [r = radius of measuring tube (m)] (m^3)
C	=	correction factor for gas expansion as a result of change in static head and viscosity of water.
L	=	length of wood specimen (m)
P_{atm}	=	atmospheric pressure (m Hg)
$\bar{z}$	=	average height of water over surface of reservoir during period of measurement (m)
t	=	time (s)
A	=	cross-sectional area of wood specimen (m^2)
Δz	=	change in height of water during time t (m)
V_r	=	total volume of apparatus above point 1 (including volume of hoses) (m^3)

Vessel Properties Measurement

Disks were taken from the trees at breast height (1,3 m above the ground level) and then air-dried. A pith to bark strip of about 1 cm wide and 1,5 cm thick was cut along the average disc diameter; the strip was used for measuring vessel attributes. After soaking in hot distilled water for 24 hours, the strip was cut to its individual tree rings with a sharp chisel. Cross sections, 15 μm thick, were cut from these samples with a sliding microtome, stained with safranine, dehydrated in a graded alcohol series, and mounted in Canada balsam C-1795 (a mounting resin medium for microscopy). Four fields in each direction (maximum and minimum growth rate directions) were selected for light microscopic analysis, and thus eight fields from each growth ring were measured. Each growth ring was subdivided into four zones from pith to bark in each direction. Each zone covered 20% of the distance between the start of the earlywood up to the end of latewood. The number of vessels in 0.4 mm^2 fields was counted. The maximum and minimum VLDs (vessel lumen diameter) for all individual vessels in each field were measured and averaged. Depending on the treatment and the position of the growth ring, the number of vessels in each field varied from ca. 20-50. VLA% (percentage of vessel lumen area) was then calculated based on VF (vessel frequency) and VLD in each field.

Nanosilver Impregnation

A 200 ppm aqueous dispersion of silver nanoparticles was produced using electrochemical technique. The size range of nano-suspension was 10 to 80 nm. The pH of the suspension was 6- 7; two kinds of surfactants (anionic and cationic) were used in the suspension as stabilizer; the concentration of the surfactants was three times the metal nanoparticles. Empty-cell impregnation process (Rueping method) was carried out under 2,5 bar pressure for 20 minutes. After the impregnation, all the impregnated specimens were kept at room conditions (30±2°C, and 45±3% relative humidity) for 4 months. Specific gas permeability values of all specimens were again measured before heat-treatment process. Due to the empty-cell impregnation process, the nano-suspension was absorbed by the cell wall and therefore the nanoparticles were stuck to the cell wall once the carrying water was evaporated. Furthermore, the high depth of penetration to the deepest parts of the specimens made the nanoparticles impossible to be wiped out by abrasion.

Treatment in Vaporized Chamber

Once all the longitudinal specimens (un-impregnated and nanosilver-impregnated) were kept together in room conditions for four months, they were put in a vaporized-chamber (43±2°C, and 80±3% relative humidity). Specific gas permeability was measured every month, for six consecutive months. When the vaporized chamber was opened each month, replications of each treatment were kept in plastic bags to prevent exchange of moisture with the surrounding atmosphere; each specimen was then brought out for measurement. No precautions were made to restrain or prevent growth of molds and fungi so that not to alter the conditions in real nature.

SEM Imaging

Scanning Electron Microscope (SEM) imaging was carried out at thin-film laboratory, FE-SEM lab (Field Emission), School of Electrical & Computer Engineering, The University of Tehran; a field-emission cathode in the electron gun of a scanning electron microscope provides narrower probing beams at low as well as high electron energy, resulting in both improved spatial resolution and minimized sample charging and damage.

Statistical Analysis

Statistical analysis was conducted using SAS software program, version 9.2 (2008). Two-way analysis of variance (ANOVA) was performed to discern significant difference at the 95% level of confidence. Grouping was then made between treatments using the Duncan test. Hierarchical cluster analyses, including dendrogram and using Ward methods with squared Euclidean distance intervals, were carried out by SPSS/18 (2010). Regression graphs were made by Minitab software, version 16.2.2 (2010).

RESULTS

Impregnation with Metal Nano-Particles

Nano-silver adsorption was 461 and 435 kg/m^3 in outer-wood and inner-wood, respectively. Statistically, no clear difference was observed between NS-adsorption in the treatment with and without alfalfa.

Longitudinal Gas Permeability

Maximum specific longitudinal gas permeability was observed in the nanosilver-impregnated inner-wood specimens of the treatment with alfalfa (1614.4 × 10^{-13} m^3 m^{-1}), and minimum permeability was found in the un-impregnated outer-wood specimens of the treatment without alfalfa (491,7 × 10^{-13} m^3 m^{-1}) before the vaporization (Figure 2). Nanosilver impregnation increased specific gas permeability in all treatments; maximum increase was found in the inner-wood specimens of the treatment with alfalfa (95,8 % of increase).
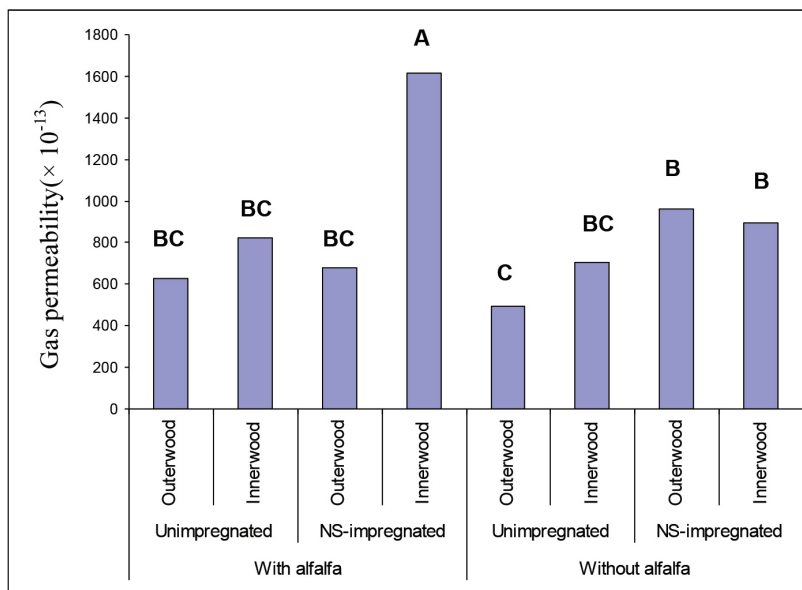
Figure 2. Specific longitudinal gas permeability values for the inner- and outer-wood in the two treatments of with and without alfalfa, both treatments with 3× 4 m initial spacing ($\times 10^{-13}$ m³ m⁻¹).
(NS=NanoSilver; Innerwood=wood produced in the first five years of growth; Outerwood=wood produced in the last five years of growth)
(Capital letters on each column are the Duncan groupings).

Radial Gas Permeability

Specific radial gas permeability values were significantly different in the inner and outer-woods (Figure 3). The first five years of growth (the inner-wood) showed higher permeability in both treatments of with and without alfalfa. The difference between the inner and outer-woods was higher in the treatment with alfalfa. The highest radial permeability was observed in the inner-wood of treatment with alfalfa ($0,364 \times 10^{-13}$ m³ m⁻¹); and the lowest permeability was $0,293 \times 10^{-13}$ (m³ m⁻¹), found in the outer-wood of the treatment without alfalfa.
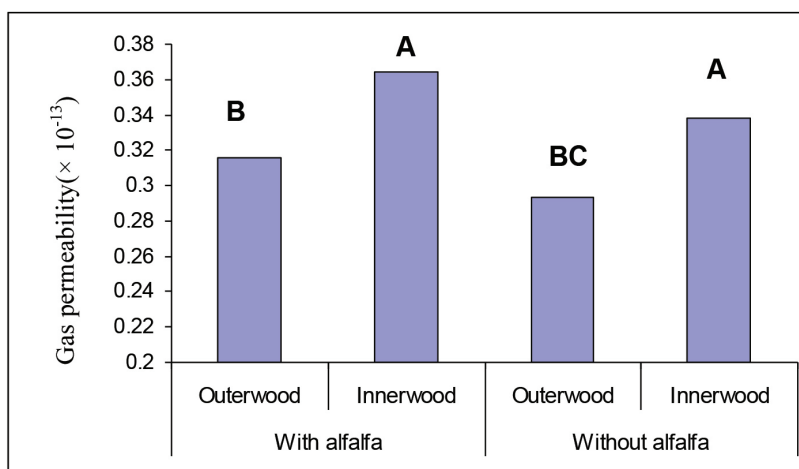

Figure 3. Specific radial gas permeability values for the inner- and outer-wood in the two treatments of with and without alfalfa, both treatments with 3×4 m initial spacing ($\times 10^{-13}$ m³ m⁻¹).
(Innerwood=wood produced in the first five years of growth; Outerwood=wood produced in the last five years of growth)
(Capital letters on each column are the Duncan groupings).

Vaporized Chamber

Vaporized chamber increased gas permeability at the first phase of measurement (one month) in all treatments but the outer-wood nanosilver-impregnated specimens of the treatment without alfalfa (Figure 4). Gas permeability was then decreased in the fourth consecutive phases; in the sixth phase, gas permeability increased again.
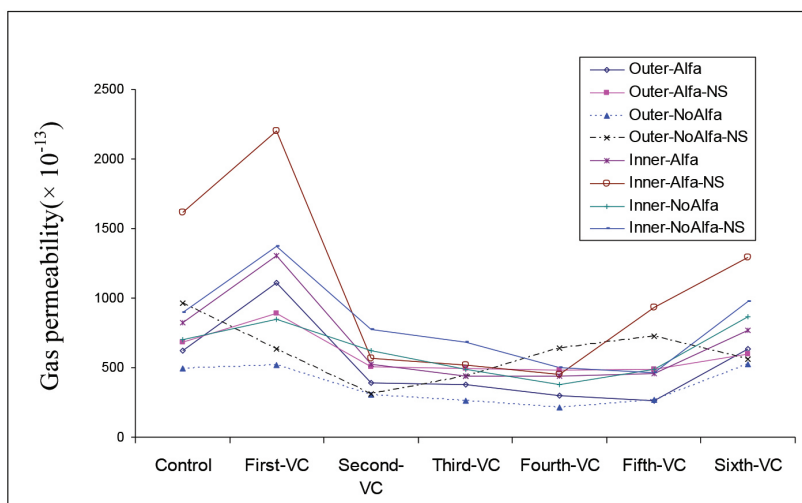

Figure 4. Specific longitudinal gas permeability values for the inner- and outer-wood in the two treatments of with and without alfalfa kept in a vaporized chamber for six months (43±2°C, and 80±3% relative humidity), both treatments with 3 × 4 m initial spacing (× 10^{-13} m³ m^{-1}).
(NS=NanoSilver; Inner=wood produced in the first five years of growth; Outer=wood produced in the last five years of growth; Alfa=treatment with alfalfa; NoAlfa=treatment without alfalfa).

DISCUSSION

Intercropping poplar trees with alfalfa, nitrogen-fertilizers, and wider initial-spacing were reported to improve vessel properties and fiber attributes (Efhami *et al.* 2010, 2012; Taghiyari *et al.* 2011; Arnold and Mauseth 1999), and consequently, it generally increased gas permeability. The results of the present study approved the effects of intercropping with alfalfa on improvement of all vessel properties by alfalfa-intercropping. Scatter-plots drawn between the specific gas permeability with the three vessel properties showed positive correlation between all of them (Figure 5). Significant increase was also observed in VLD (vessel lumen diameter) and VLA (vessel lumen area) of the second five year of growth of the trees; however, vessel frequency (VF) was decreased. Results also indicated that inner-wood (the first five years of growth) of both treatments had higher permeability than the outer-wood (the last five years of growth). This was in contradiction with previous studies (Taghiyari and Efhami 2011, Taghiyari *et al.* 2011) that reported the inner-wood (heartwood, and juvenile wood) to be less permeable than that of the outer-wood. In this connection, permeability in solid woods is considered a function of accessibility to the lumen and cell wall cavities; therefore, slight changes in the vessel properties and pit structure, and even micro-cracks and fissures in the cell wall, would result in a significant increase in the specific air permeability (Taghiyari *et al.* 2012c); that is, if each of the vessel properties (vessel lumen area, vessel lumen diameter, and vessel frequency) alters slightly, it would result in a significant change in the fluid transfer process in wood (Taghiyari and Efhami 2011, Taghiyari 2013). In the present study, VLD and VLA showed an increasing trend from the inner-wood to the outer-wood (Efhami *et al.* 2010, Efhami and Saraeyan 2009) (Table 1), therefore it was anticipated that the gas permeability would increase. However, longitudinal and radial permeability of the inner-wood (near the pith) were higher, in both

treatments of with and without alfalfa (Figure 2 and 3). The reason was the infestation of the inner-wood with fungi during the growth of the trees. In this connection, fungi growth was reported to proliferate within the pit membranes and primary cell walls of ray parenchyma cells in pine wood, resulting in an increase in permeability of sapwood (Woo *et al.* 2005).
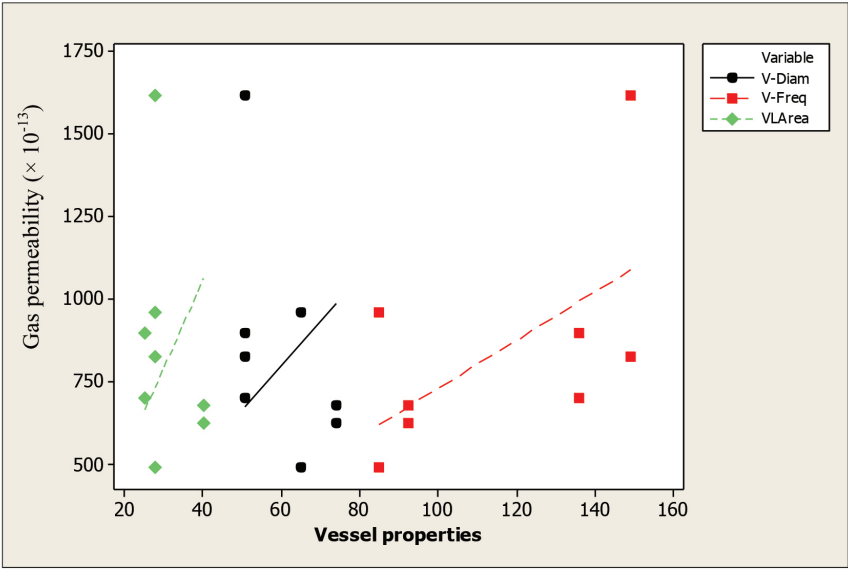

Figure 5. Scatter-plot of the specific gas permeability versus the three vessel properties of vessel diameter (V-Diam), vessel frequency (V-Freq), and vessel lumesn area (VLArea).

Table 1. Vessel properties in the inner-wood (first five years of growth) and outer-wood (second five years of growth) in the two treatments of with and without alfalfa, both with 3 m × 4 m of initial spacing.

Vessel properties	Inner-wood (first five years of growth)		Outer-wood (second five years of growth)	
	With alfalfa	Without alfalfa	With alfalfa	Without alfalfa
Vessel diameter (μm)	50,7	50,6	73,7	64,9
Vessel frequency (per mm)	149	136	92,4	84,8
Vessel lumen area (%)	27,7	25,1	40,1	27,8

Nanosilver-impregnation resulted in a significant increase in the longitudinal gas permeability due to the dissolving of extractives and pitch deposits that blocked pits and perforation plates (Koch 1996, Rice and D'onofrio 1996), as well as tearing of vessel perforation plates (Taghiyari 2012a); both phenomena caused easier fluid transfer and a consequent increase in the specific gas permeability (Figure 6).
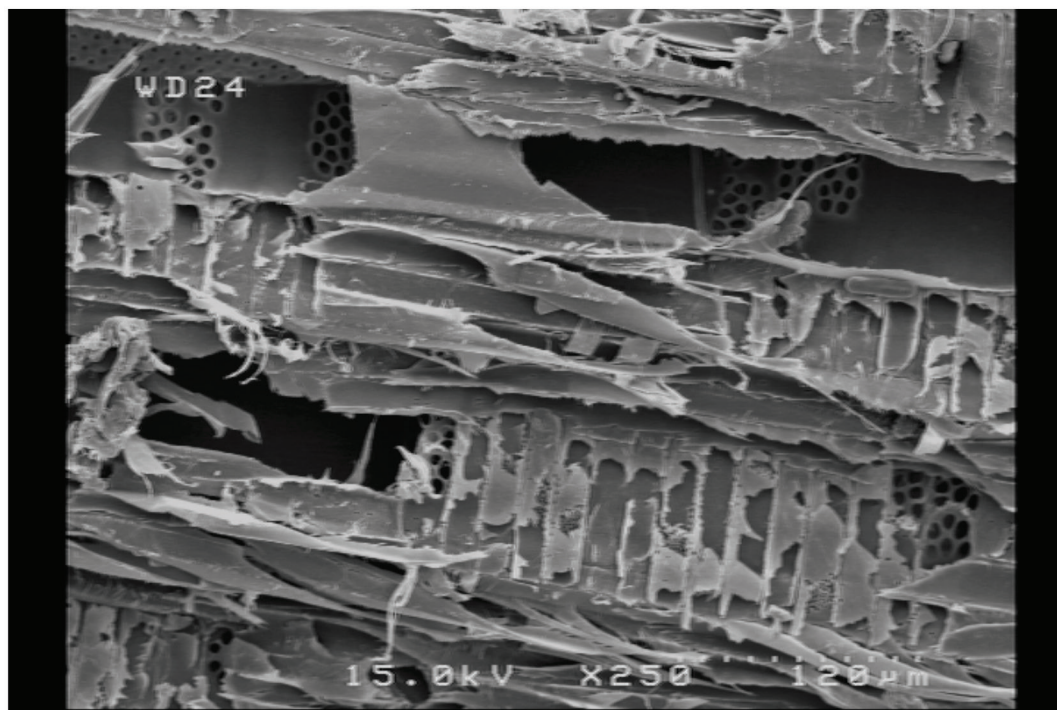

Figure 6. SEM image showing perforation plates torn out due to the high pressure in the nanosilver-impregnation vessel (× 250).

Nearly all treatments showed an increase in permeability at the first stage of vaporized chamber (Figure 4). This increase was due to the initial temperature alteration on the specimens; that is, the temperature was fixed at 43±2°C whereas the ambient temperature was about 22°C. This resulted in more loss of water than gain in the specimens at the initial stages, resulting in shrinkage and wider vessels; ultimately, the fluid transfer was easier. As the process of vaporizing continued, water vapor penetrated into the specimens and absorbed by the cell wall, resulting in swelling and the consequent decrease in permeability. This process continued until the last permeability measurements (the 5th and 6th vaporized chamber phases) in which fungi and molds had enough time to grow and re-grow, to further deteriorate the pits and perforation plates, resulting in an increase in permeability despite of the conditions that facilitated more gain of water vapor and swelling. All treatments showed nearly the same trends in fluctuations in permeability values; this implied that the antibacterial property of silver nanoparticles that were spread through the cell walls did not show significant difference in the growth of molds and fungi (Figure 7); it may therefore be concluded that 200-ppm nanosilver dispersion can not significantly limit the deteriorating effects of molds and fungi molds and fungi in poplar.
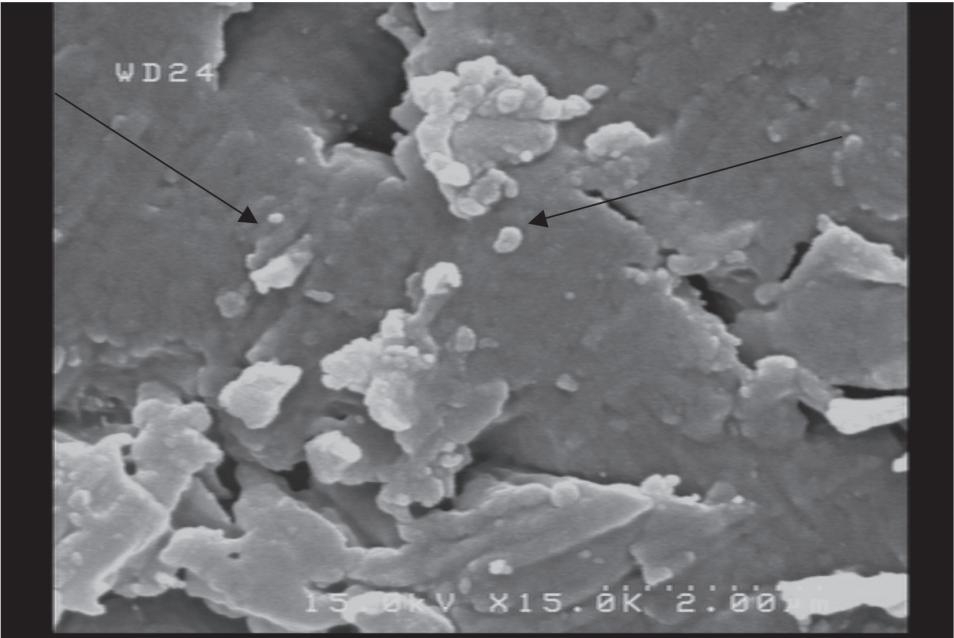
Figure 7. SEM image showing silver nanoparticles spread all over cell walls (× 15,000).

Cluster analysis based on all vessel properties and gas permeability values (at the initial stage of measurement, as well as six measurements while kept in vaporizing chamber) showed that inner- and outer-woods were clustered quite differently (Figure 8). This proves that all properties in relation with the fluid transfer in poplar wood were significantly affected by the age of the wood produced. Furthermore, in all sub-groups, control treatments and those impregnated with nano-silver suspension were closely clustered; this again may indicate that 200-ppm nanosilver can not be considered a suitable level for limitation of molds and fungi.
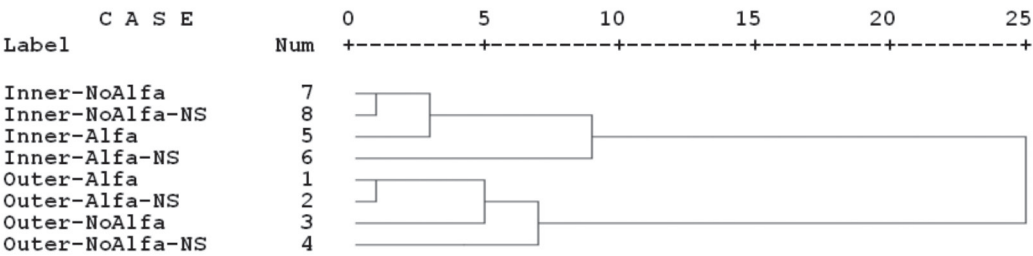

Figure 8. Cluster analysis of the eight different treatments (NS=NanoSilver; Inner=wood produced in the first five years of growth; Outer=wood produced in the last five years of growth; Alfa=treatment with alfalfa; NoAlfa=treatment without alfalfa).

CONCLUSION

The present study confirmed that intercropping with alfalfa has an increasing effect on the specific gas permeability both in the longitudinal and radial directions of downy black poplar. This increase was due to the improvement of vessel properties caused by nitrogen enrichment in the soil of the trees intercropped with alfalfa. Furthermore, infestation by fungi during the growth of the trees proliferated pits and vessel perforations, resulting in easier transfer of fluid and higher permeability; therefore, higher permeability was observed in the first five years of growth (inner-wood) than the last five years (outer-wood), although two vessel properties (vessel lumen diameter and vessel lumen area) were in favor of the outer-wood to be more permeable. Long-term vaporization of specimens also facilitates the growth or re-growth of molds and fungi, resulting in easier transfer of fluids. Intercropping with alfalfa as an N-fixing plant may both satisfy agricultural needs as well as provide wood with higher permeability for impregnation and preservation industries. Young poplar stands are more susceptible to infestation and attack by fungi and deterioration agents. Therefore, precautions are recommended to be taken to prevent infestation by fungi during the growth of the trees, eliminating the negative effects on physical and mechanical properties of the wood produced.

ACKNOWLEDGMENT

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