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DECAY RESISTANCE OF PRESERVATIVE INJECTED POPLAR AND ITS PROCESS OF PREVENTING WHITE ROT FUNGI INFECTION

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ABSTRACT

This study initially investigated decay resistance of preservative injected poplar and its infection mechanism of preventing white rot fungi. The living poplar was injected with different concentrations (0.0, 0.5, 1.0 and 1.5 wt.%) of alkaline copper quaternary (ACQ). Using the scanning electron microscopy, the process of preservative injected poplar wood preventing white rot fungi infection at different tree heights were examined. The decay resistance test results showed that the decay resistance of preservative injected poplar at different tree heights was significantly different. With the increase in tree height, the decay resistance decreased, and the higher the ACQ concentration, the better the decay resistance. The white rot fungi infested the poplar wood with the vessels as the breakthrough point, being spread to other cells through pits. The white rot fungi degraded wood cell walls by secreting enzymes and preferentially degraded the parenchyma cells.

KEYWORDS: Poplar wood, living trees preservation, decay resistance, white rot fungi.

INTRODUCTION

Poplar has short growth cycle and large yield, but the drawbacks of low density, soft texture, easy discoloration, and perishable decay have limited its efficient applications. In order to

improve the durability of poplar wood, it is necessary to conduct preservation treatment on poplar wood (Griggs et al. 2017, Humar and Thaler 2017, Kukowski et al. 2017). After preservation treatment, the service life of wood products can be extended by 5-6 times. At present, wood preservation technologies mainly include spray treatment, brush treatment, immersion treatment, and pressure treatment. The spray treatment and brush treatment are only the surface treatment of the wood, and the preservation effect is only limited to treat the wood surface. Although the effect of immersion treatment is better than spray and brush treatments, the time of immersion treatment is much longer, the penetration depth of preservative is limited and retention after treatment is low, therefore, the preservation effect is not obvious (Sun et al. 2017). Pressure treatment is to allow the preservative liquid to enter the wood through external pressure. The preservative penetration depth and the retention time are improved, resulting in the increase in the preservation effect compared to the brush treatment and immersion treatment. However, the pressure treatment is more complicated, consumes a lot of energy, reduces the bending strength of the processed lumber, and often causes preservative leaching during wood service, resulting in serious environmental concerns (Rabbi et al. 2015, Tren et al. 2011, Kinata et al. 2012).

Recently, a new wood preservation method, living tree injection treatment has been used to address the problem of copper leaching during its service caused from traditional alkaline copper quaternary (ACQ) pressure-treated wood. The living trees injection treatment is different from the traditional wood preservation technologies. The preservative liquid is injected into the standing tree and goes up with the tree sap. The preservative treatment is completed when the tree is live. The living tree injection treatment requires that the preservative must be a water-soluble, diffusing preservative, and there should be no impurities such as sediment and dust. Spring and summer are the best treatment seasons (Liang 1997). This method is simple to operate, low cost, and does not affect the strength of the wood. The injection treatment is to drill holes in the trunk of a living tree and inject the preservative liquid into the trunk (Fig. 1). The liquid goes up to reach the entire tree as the tree sap rises. Some researchers used the injection treatment to input ACQ-D and borate as preservative into living poplar trees (Zhang 2013, Liu 2016), and explored the influence of climate factors on the flow rate of ACQ-D in live trees. Their results indicated that the rising rate of ACQ-D was affected by solar radiation, ambient temperature, and air humidity.

After the preservative entered the tree, its vertical transportation mainly depended on the power generated by transpiration, and the horizontal transportation depended on the osmosis between cells, which is closely related to the concentration of the preservation (Li 2013, Fan 2016). Different concentrations and different heights had different distribution patterns in the cross section (Liu 2019). At the same concentration, as the height of the poplar tree increased, the preservative distribution area of the cross-section the gradually decreased, and the preservative distribution area near the injection point was the largest, and the maximum penetration area reached 50% of the cross-section of the trunk. The preservative distribution area was reduced far away from the injection point. Because in the continuous injection process of the tree, the place near the injection port was always in contact with the preservative, and the preservative penetrates laterally continuously. The place away from the injection point until

the preservative rises to this position before the horizontal penetration. The fixation part of preservative in wood was mainly the cell walls. There are many pore structures of different sizes on the poplar cell walls (Liang et al. 2020). The larger the cell pores, the easier it is for the preservative to be fixed. At the same height cross section, the higher the preservative concentration, the smaller the distribution area, but the higher the preservative retention; the lower the preservative concentration, the larger the distribution area, but the lower the preservative retention. The trend of preservative retention at different heights of the trunk was similar to the distribution area of preservative. On the same height, the higher the concentration of preservative, the higher the preservative retention of poplar. The penetration area and preservative retention changed due to concentration and height. Therefore, it is necessary to evaluate the decay resistance of preservative injected poplar trees by measuring the mass loss produced in the decay resistance test, and to explore the decay resistance difference in the tree height.

The wood decay resistance test is a method to determine the wood decay resistance grade by infecting wood by decay fungi and causing mass loss under laboratory conditions. The main components of wood cell walls, cellulose, hemicellulose, and lignin are the main sources of nutrients for decay fungi (Eastwood et al. 2011, Floudas et al. 2012, Riley et al. 2014). Wood decay can be divided into brown rot, white rot, and soft rot. Although brown rot and white rot can occur on softwood and hardwood, it is generally believed that brown rot mainly occurs on softwood and white rot mainly occurs on hardwood (Schwarze et al. 2003, Kaffenberger and Schilling 2014, Zhang et al. 2019). The white rot fungi can degrade both cellulose and lignin, mainly through side chain oxidation and aromatic ring cleavage (Brai et al. 2019). The main methods of white rot fungi to decay wood are simultaneous rot and selective delignification. The simultaneous rot occurs near the hyphae, and the enzymes secreted by the hyphae degrade cellulose, hemicellulose, and lignin at almost the same rate. For the selective delignification, lignin has priority over cellulose and hemicellulose degradation (Schwarze 2007, Blanchette et al. 2010, Bari et al. 2017, Brai et al. 2019, Bari et al. 2020). Simultaneous rot is the main type of white rot. Simultaneous rot and selective delignification can be caused by the same fungi, and the two processes often occur simultaneously (Krishna et al. 2007, Sonam et al. 2012). For the poplar white rot, simultaneous rot and selective delignification often happen at the same time. The white rot fungi preferentially degrade parenchyma cells (Barry et al. 2008, 2017, Sunardi et al. 2018).

In this study, ACQ-D with different concentrations were injected into live poplar trees to achieve wood preservation purpose. Using a unique technology of inhibiting the white rot fungi growth and enzyme activity through experiments, the decay resistance tests were conducted on the wood samples obtained from different poplar tree heights, the mass losses were measured at different time intervals, and the process of preservative injected poplar wood preventing white rot fungi infection was analyzed. The findings and results of this study can provide a theoretical basis of the living tree preservation mechanism.

MATERIALS AND METHODS

Materials

Twelve 10-year-old living poplar trees (*Populus* × *euramericana* Nanlin 895) without physiological defects and in good growth conditions were adopted by the three-hole injection method (the injection points were at a height of about 20 cm, the angle with the longitudinal axis of the tree trunk was about 45°), in a Forest Farm at the Nanjing Forestry University, Nanjing, Jiangsu Province, China. The preservative was ACQ-D. The poplar trees were divided into four groups, three trees in each group, and the preservative concentrations were 0 wt.% (representing the control group, that is, the untreated poplar in the text), 0.5 wt.%, 1.0 wt.%, and 1.5 wt.%, resp. The injection volume was 2500 mL. After the injection was finished, the trees were fallen. Wood samples with a thickness of about 2 cm were cut from the vicinity of the injection port of each group of trunks upwards every 2 m (Fig. 1). The samples were respectively marked as T0, T2, T4, T6, T8 and T10, where T0 was the sample near the injection point, T2 was 2 m away from the injection point, T4 was 4 m away from the injection point, T6, T8, and T10 were 6 m, 8 m, and 10 m from the injection point (Fig. 1).

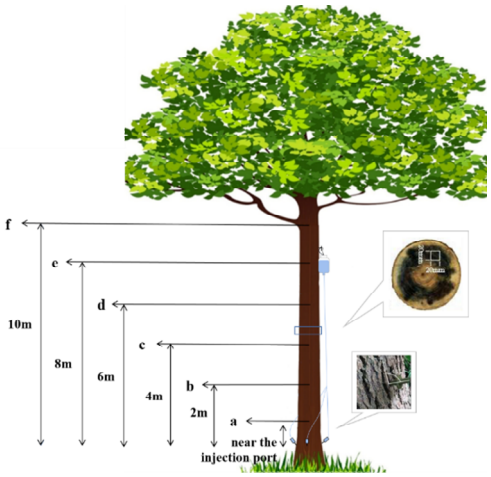


Fig. 1: A living tree preservation diagram and cross-sectional sampling at different heights.

Decay resistance test

(1) Sample preparation: Poplar wood was cut into samples with a size of 20 mm (R) × 20 mm (T) × 10 mm (L). Eighteen samples with different preservative concentrations from different tree heights were divided into three groups, counting six in each group. *(2) Preparation of maltose agar culture medium:* Maltose (°Bé 1.03) and agar were used to configure the medium in the petri dish, and then fungi were inoculated after sterilization. The test fungi was *Coriolus versicolor* (L. exFr) Quel (strain number: CFCC5336, from China Forestry Culture Collection Center (CFCC), Beijing, China), which was placed in an incubator (at a temperature of 28 ± 2°C and relative humidity of 80%) for 7 days. *(3) Preparation of river sand and sawdust culture medium:* The dried river sand, poplar sapwood sawdust, corn meal, and brown sugar were added into a culture bottle with evenly stirring. Then the glucose solution was added into the culture bottle. The feeding wood samples were placed on the surface of the culture medium,

and then sterilized the whole culture bottle. *(4) Fungi inoculation:* The mycelium block was cut in the petri dish and connected to the river sand culture medium (about 5 mm deep on the surface of the culture medium). The whole operation was carried out under aseptic conditions, and then the samples were placed in an incubator for 7-10 days. *(5) Samples were decayed by fungi:* Each sample was numbered, placed in an oven at a temperature of 103 ± 2°C to be dried to a constant weight (accurate to 0.01g), wrapped in moisture-absorbing paper, and placed in a steam sterilizer for sterilization. When the samples reached 40% - 60% moisture content, the sterilization was stopped, and the samples were cooled to room temperature. Under aseptic conditions, the fungi were infested on the feeding wood samples that had been overgrown with decaying fungi and placed them in an incubator. *(6) Mass loss detection:* To monitor the decay process, the samples were taken out from the incubator after the experiments of 4, 8 and 12 weeks for measurements. After the hyphae and impurities on surfaces were gently scraped with a blade, the samples were dried to a constant weight in an oven at 103 ± 2°C, weighed each separately, and calculated the mass loss according to the following Eq. 1:

$$\text{Mass loss} = (\text{W1} - \text{W2})/\text{W1} \times 100\% \quad (1)$$

where: W1 - the oven-dry weight of the sample before the test; W2 - the oven-dry weight of the sample after the test.

According to the standard of GB/T13942.1-2009 *Durability of wood-Part 1: Method for laboratory test of natural decay resistance*, the mass loss of the sample with an experiment time of 12 weeks was evaluated for decay resistance.

Tab. 1: Evaluation standard of wood decay resistance classification.

Durability	**Mass loss of hardwood**
Very resistance	1-10%
Resistance	11-24%
Moderately resistance	24-44%
Non-resistance	> 45%

SEM observation

When the test time durations were 4, 8 and 12 weeks, the samples were taken out to measure their mass losses. T0 samples with different concentrations were chosen for observations. The samples were cut apart, and the part that was not in direct contact with the decay fungi was selected, cut them into 20 μm thick section slices and absolutely dried. Sprayed gold with a vacuum coater, and then used Quanta 200 SEM (FEI USA) to observe the white rot fungi infection process. The selected acceleration voltage was 15 kV and the working distance was 13 mm.

RESULTS AND DISCUSSION

A 12-week decay resistance test was conducted, the mass losses were measured, and the decay resistance grade were evaluated according to the standard of GT/T13942.1: 2009 (Fig. 2). It can be seen in Fig. 2 that the untreated poplar was not resistant to decay, with a mass loss of 45.51%, and the mass losses of all ACQ-injected poplar samples were less than that of the untreated poplar. The wood samples taken from lower heights, namely T0, T2, T4, had low mass losses and high decay resistances. Except for the samples of T4 with 0.5 wt.% ACQ-D concentration, they all reached the 'Resistance' grade, and the poplar wood with 1.5 wt.% ACQ-D concentration reached the 'very resistance' grade, showing the mass loss of only 8.72%. The mass losses of the samples with different ACQ-D concentrations in the T6 section had a greater increase, and the decay resistance grade was 'moderately resistant'. At the same height, the higher the concentration, the better the decay resistance.

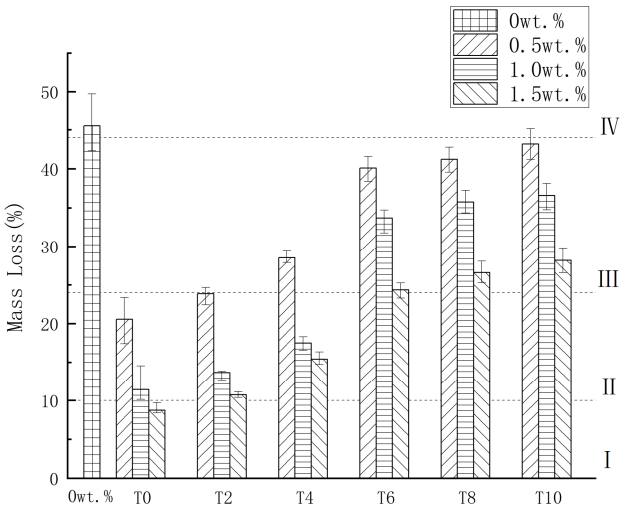


Fig. 2: The mass loss of preservative injected poplar.

In addition to analyzing the mass loss in 12 weeks, the mass losses at 4 and 8 weeks of infection were measured and analyzed by combining SEM (Fig. 3-5). When the infection time was 4 weeks, the white rot fungi spread through the pits on cell walls, the cell walls were not significantly damaged, and the mass loss of the sample only accounted for a small portion of the 12-weeks mass loss. When the infection time was 8 weeks, the ray parenchyma cell walls were damaged, and the mass loss of the sample increased. After 12 weeks, the walls of the wood vessels and the wood fibers had holes, and the mass loss increased significantly.

Decay resistance analysis

The wood samples from different tree heights had different ACQ loadings, resulting in different preservative treatment results for the wood from different sections of the ACQ injected tree (Fig. 1). The preservative treatment results from the ACQ injection for different heights of the living tree can be determined by examining the wood decay resistance, which can be determined by measuring the mass loss during the decay test. Six wood samples were obtained from different heights of the injected tree, i.e., Point T0 (near the injection point), and Point T2 (2

m from the injection point), Point T4 (4 m), Point T6 (6 m), Point T8 (8 m), and Point T10 (10 m) as shown in Fig. 1. According to the national standard of *Durability of wood - Part 1: Method for laboratory test of natural decay resistance*, a 12-week decay resistance test was conducted, and the result is shown in Fig. 2.

In order to analyze the influence of ACQ concentration and different heights along the tree on mass loss, the Duncan's multiple range test (DMRT) was utilized to measure specific differences between pairs of means. DMRT was originally proposed by Duncan (1975) as a higher-power alternative to Newman–Keuls. DMRT is more useful than the Least Significant Difference (LSD) when larger pairs of means are being compared, especially when those values are in a table (Shafaei and Kamgar 2017). Tab. 2 shows the mass loss (%) of the samples from different heights of the tree (Fig. 1). To compare the difference of the decay resistances at different tree heights, DMRT was used, and the results are presented in Tab. 2. The groups with the same letters in each column indicate that there is no statistical difference ($p < 0.05$) between the samples according to DMRT. The results showed that, when the ACQ concentrations were 1 wt.% and 1.5 wt.%, the mass loss significantly increased gradually as the tree height increased from Point T0 to Points T2, T4, T6, T8 and T10 during the decay tests as shown in Tab. 2. While, when the ACQ concentration was 0.5 wt.%, no significant increases of mass loss were found from Point T0 to Point T2 and from Point T6 to Point T8 as shown in Tab. 2. This could be caused by the low ACQ concentration (0.5 wt.%) in the preservative. After the preservative entered the poplar tree, its upward flow mechanism can be explained by transpiration. The ACQ-D rose with the tree sap and was constrained by liquid gravity at the same time (Liu et al 2019a). As the height increased, the gravitational potential energy of the ACQ-D increased, the preservative was difficult to rise. The preservative attached to the trunk decreased, the mass loss increased, and the decay resistance decreased. The horizontal penetration of the preservative in the trunk was related to the concentration of the preservative. The smaller the concentration, the greater the permeability. Therefore, in the preservative injected poplar with 0.5 wt.% ACQ-D concentration, the spread of the preservative on the cross-section of the trunk was wider, also affecting the mass loss differences between Points T0 and T2, and between Points T6 and T8, which did not increase significantly.

Tab. 2: Effects of ACQ concentration and different heights of tree on the mass loss (%).

Height of tree	**ACQ concentration**		
	0.50%	1.00%	1.50%
T0 (Near the injection point)	20.56 (3.59) a	11.46 (2.15) a	8.72 (0.26) a
T2 (2 m)	23.84 (1.26) a	13.65 (0.24) b	10.72 (0.23) b
T4 (4 m)	28.53 (1.38) b	17.47 (0.06) c	15.39 (0.29) c
T6 (6 m)	40.08 (2.72) c	33.68 (0.48) d	24.34 (0.47) d
T8 (8 m)	41.20 (0.45) c	35.74 (1.04) e	26.60 (0.64) e
T10 (10 m)	43.16 (0.64) d	37.59 (1.24) f	28.18 (0.41) f

*Values in parentheses are standard deviations. Groups with the same letters in each column indicate that there is no statistical difference ($p < 0.05$) between the samples according to the Duncan's multiple range test.

Tab. 2 indicates that the mass loss was the largest for the sample at the tree height of 10 m (point "*f* " in Fig. 1). Thus, the mass losses (%) between three types of ACQ-injected samples at

the tree height of 10 m (point f) and control samples were compared and the results are listed in Tab. 3. As shown in Tab. 3, the mass losses of the control were significantly larger ($P < 0.05$) than the samples injected with 1.0 and 1.5 wt.% ACQ, and the mass losses of the control were significantly larger ($P < 0.1$) than the samples injected with 0.5 wt.% ACQ. The results of decay tests revealed that, compared to the untreated control samples, the decay resistance of the ACQ-injected samples was significantly improved, even the samples obtained from the highest position of the injected poplar tree trunk (point "*f* "). The mass loss of poplar with 0.5 wt.% ACQ-D concentration was more than that of 1.0 and 1.5 wt.%. This is because the concentration of preservatives is too small (0.5 wt.%), and the effective components of the preservatives fixed at the height of 10 m (T10 point) were less, so its decay resistance was close to that of un-injected poplar wood.

Tab. 3: Comparisons of mass loss (%) of ACQ-injected samples (point "f") with the control.

Sample	Mass loss (%)		*F*	*P-value*	*F crit*
	Average	Sd.			
Control	45.51	3.08			
0.5 wt.% ACQ	43.16	0.64	3.3275	0.0981	4.9646
1.0 wt.% ACQ	37.59	1.24	34.0879	0.0002	4.9646
1.5 wt.% ACQ	28.18	0.41	186.4042	0.0000	4.9646

Analysis of the process of preventing white rot fungi from infecting poplar wood

The mass losses of preservative injected poplar wood of different ACQ concentrations are shown in Fig. 3. During the test, as time increased, the mass loss of all samples also increased. The mass loss in the later stage of the test increased greatly, and the mass loss of the untreated poplar wood was the largest in the later stage of the test. The reason was that the untreated poplar wood was 'Non-resistance' grade, and there was no inclusion inside the wood that can prevent decay fungi from infecting wood. Inside the untreated poplar wood, decay fungi multiplied and increased over time, and gradually invaded the wood cells. The contact area with wood increased and the growth of white rot fungi and the degradation of wood was vigorous, resulting in a significant increase in mass loss. For the preservative injected poplar wood, the growth activity of white rot fungi and enzyme activity were inhibited by preservative (Sedris 2011, Noll et al. 2019). Therefore, the growth of mass loss of the preservative injected poplar wood was more stable. The growth of the mass loss of T6, T8, and T10 samples with poor decay resistance in 12 weeks was also higher than that of the T0, T2, and T4 samples. Because the preservative retentions in T6, T8, and T10 samples were reduced, and the inhibitory effects on white rot fungi were weakened, resulting in a larger increase in mass loss after the increase in the number of white rot fungi in the later stage.

In the untreated poplar wood, the white rot fungi infected wood cells firstly from the cell cavity of vessels and proliferated in large quantities inside the large vessels, also spread to other cells using the vessels as a breakthrough point (Francis 2007, Bari 2015). This phenomenon was suppressed in the preservative injected poplar wood.

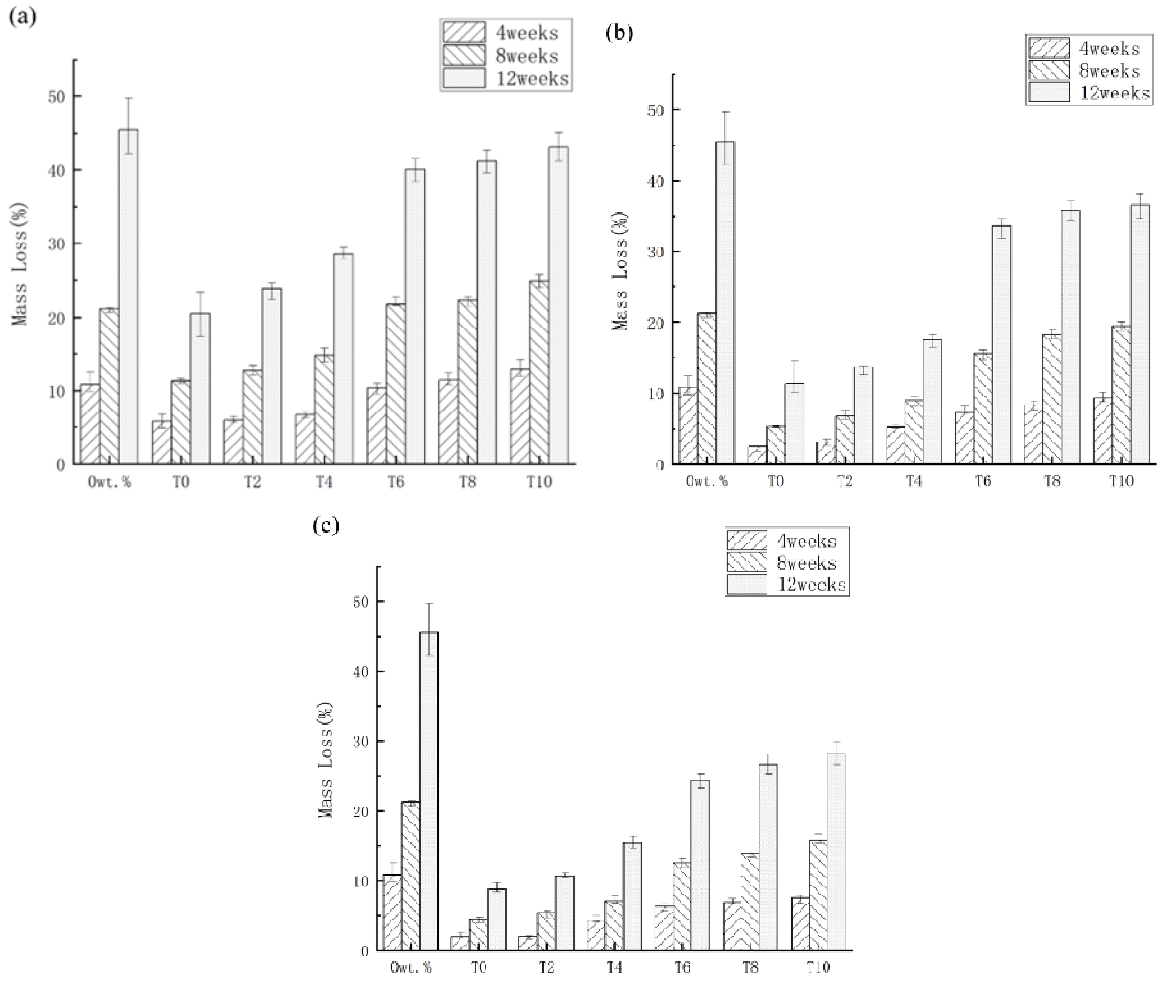
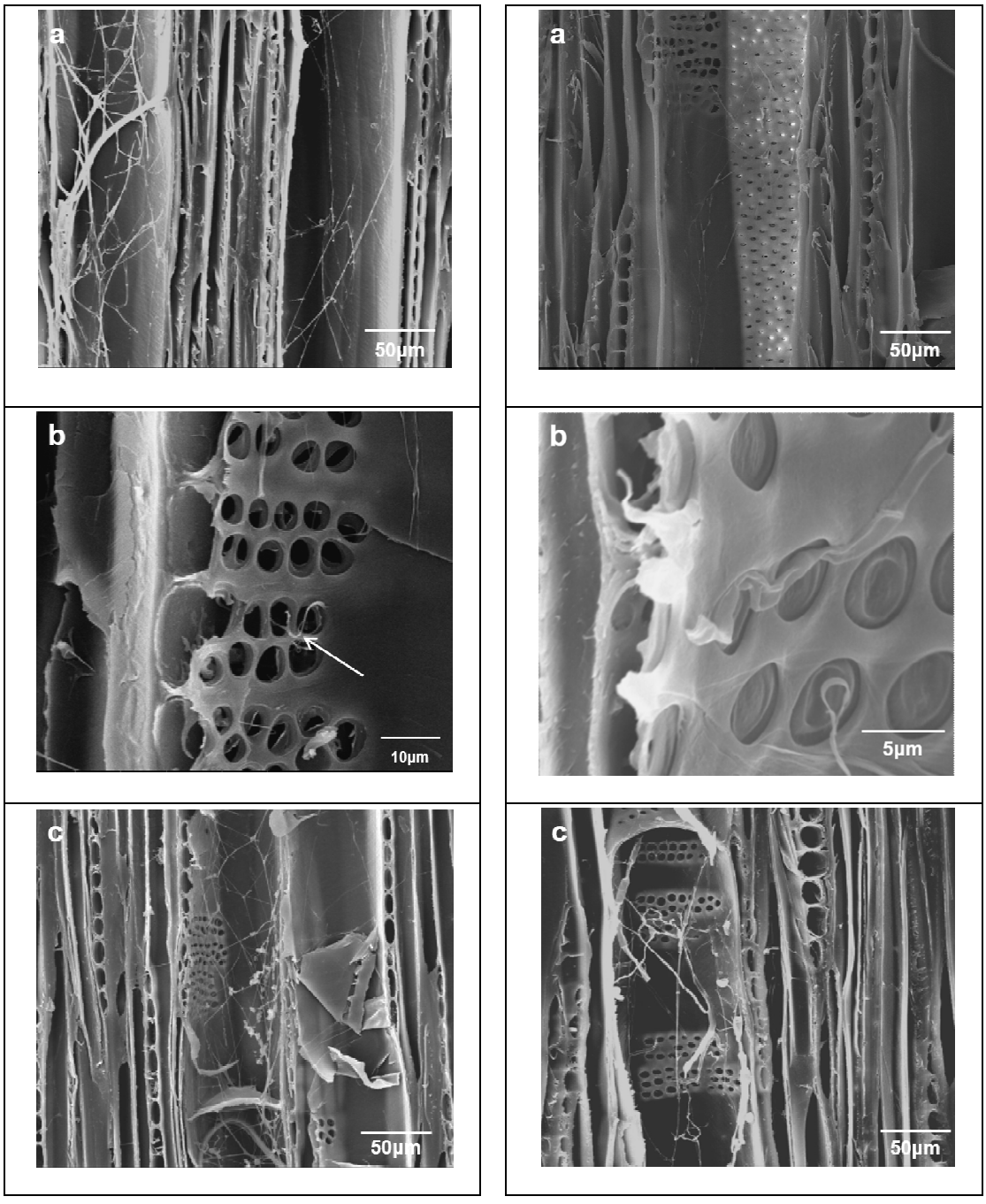
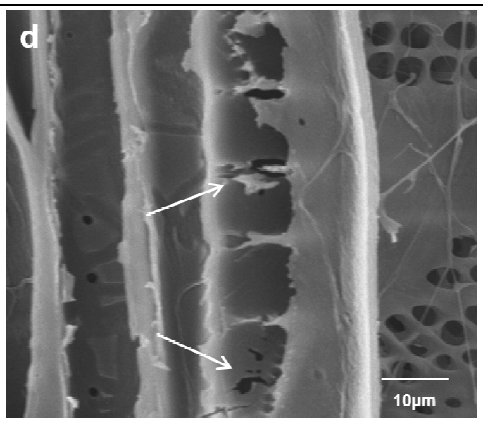


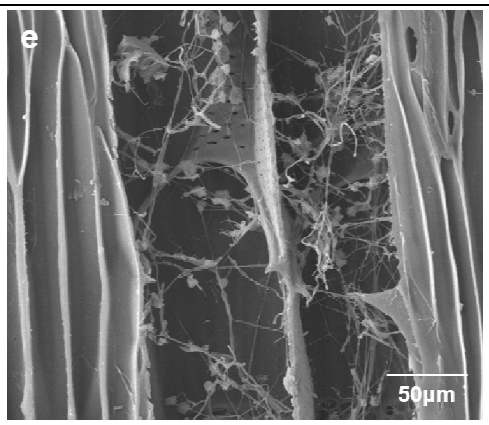
Fig. 3: Comparison of mass loss of preservative injected poplar and untreated poplar: a) 0.5 wt.% ACQ, b) 1.0 wt.% ACQ, c) 1.5 wt.% ACQ.

When the infection time was 4 weeks, a large number of hyphae can be seen in the vessels of the untreated wood and spread to other cells through pits. The hyphae destroyed the weakened parts of cell walls such as the pits through direct contact (Figs. 4a,b). In the preservative injected wood, the number of hyphae was much smaller than that of the untreated wood, and it was observed that the pits were attached to but not damaged by the hyphae, and the growth status of hyphae was poor (Figs. 5a,b). After 8 weeks' infection, the number of hyphae in the vessels of the untreated wood increased. Although the cell walls of the ray cells did not directly contact with the hyphae, they were also destroyed, and holes and cracks appeared (Figs. 4c,d). The reason was that the white rot fungi degraded the cell walls, intercellular layers, and corner of the cells which were far away from the hyphae by diffusing extracellular enzymes (Schwarze et al. 2003, Zhang et al. 2011). A small number of hyphae appeared in wood fibers. In the preservative injected wood, the number of white rot fungi was still less than the untreated wood, and the wood ray cell walls were not significantly damaged (Figs. 5c,d). After 12 weeks' infection, the number of white rot fungi in the untreated wood continuously increased, and holes appeared in cell walls of vessels and wood fibers (Figs. 4e,f) The number of white rot fungi in the preservative injected wood also increased, but the number was much smaller than the untreated wood,

sporadic hyphae appeared in wood fibers, and wood cell walls were rarely damaged (Figs. 5e,f). In the untreated wood and preservative injected wood, the ray cells were the most degraded cells. This was because the S3 layers of vessel cell walls and wood fiber cell walls have certain resistance to decay fungi, so white rot fungi preferentially degraded parenchyma cells. In the preservative injected wood, ACQ-D was mostly fixed in the vessels and wood fibers, and the fixation amount in wood rays was less than that in the vessels and wood fibers (Liu et al. 2019b). Therefore, in the preservative injected poplar wood, the damages of the vessels and wood fibers were very small, and due to the inhibitory effect of preservative, the degree of damage to the ray cells was also lighter than that of the untreated wood.







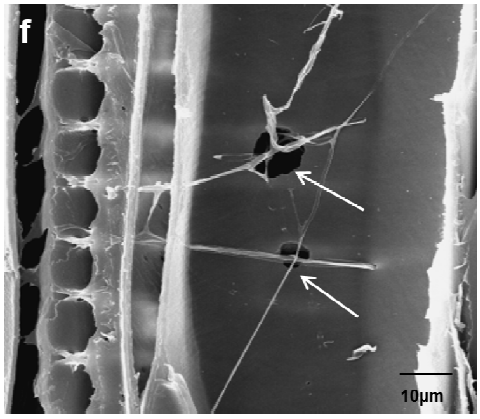
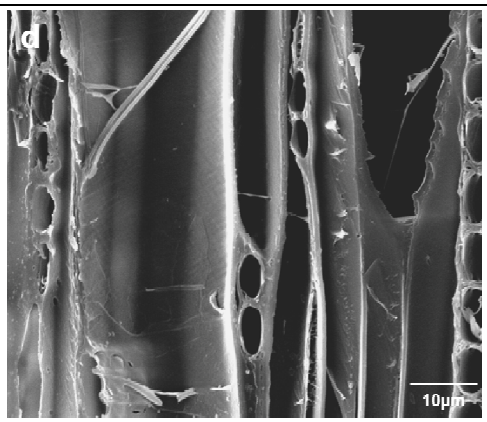
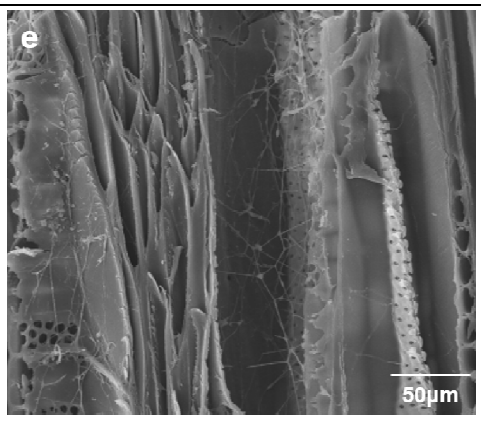


Fig. 4: The process of white rot fungus infecting untreated wood.





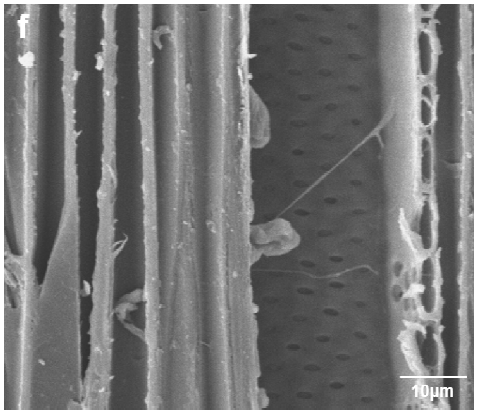


Fig. 5: The process of preventing white rot fungus infection of preservative injected poplar.

CONCLUSIONS

The decay resistance of the preservative injected poplar decreased with the increase of the trunk height. The wood samples from the near injection point had the best decay resistance. The poplar wood with 1.5 wt.% ACQ-D concentration can reach 'Very resistance' grade in the T0 section (near the injection port), with a mass loss of 8.72%, and other concentrations also reach 'Resistance' grades. The decay resistance grades of T2, T4, T6, T8, and T10 samples were reduced as the mass loss gradually increased. All treated samples reached the grade of

'Resistance' or above. The living tree injection treatment can improve the decay resistance of poplar, but there was a significant difference in decay resistance in different tree heights. The higher the height, the worse the decay resistance. During the decay process, the mass loss in the first 4 weeks was small, and the wood decayed slightly. After 4 weeks, the mass loss became larger, the wood cell walls were degraded and damaged, and the wood cells had the largest degradation of the ray cells. In the preservative injected poplar, the infection process of white rot fungi was prevented, the fungi number was reduced, and the degree of degradation of wood cells was lower than that of the untreated poplar. This study confirmed that the preservative injected poplar preventing wood decay using the unique technology of inhibiting the white rot fungi growth and enzyme activity through experiments.

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