EFFECTS OF PRESCRIBED BURNING ON PINE WOOD NEMATODE (*BURSAPHELENCHUS XYLOPHILUS)*

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Abstract. *Bursaphelenchus xylophilus* became one of the most damaging forest pests in recent years in Hunan province, and caused remarkable losses of pine tree. In order to explore the effect of fire disturbance on the prevention of *Monochamus alternatus* and *B. xylophilus.* forests dominated by *Pinus massoniana* were taken as the object of the study in Daolin town, Ningxiang city, Hunan province, China. Three kinds of forest were selected, such as healthy forest of *Pinus massoniana* (Control Ⅰ), infected forest without prevention(Ⅱ), and infected forest with prevention(Ⅲ). Then, 6 plots were set in every object forest. Three were the control plot, and the other three were treated with prescribed burning. We continuously investigated the number of *M. alternatus* and *B. xylophilus* before and after low intensity prescribed burning. The results indicated that the quantity of female *M. alternatus* in the three kinds of fire disturbance plots were reduced by 88.24%, 75.00% and 94.74%, respectively, while the males were reduced by 77.78%, 81.82% and 88.89% before and after the fire. There were significant differences for the quantity of *M. alternatus* before and after the prescribed burning ($P<0.05$). The results showed that the prevention efficacy of Ⅱ-2 was the best. Within 10 to 20 days after the fire, the quantity of *B. xylophilus* carried by *M. alternatus* changed significantly. The average quantity of *B. xylophilus* carried by female and male *M. alternatus* dropped from 1135 to 6 and from 397 to 35, respectively. It showed that prescribed burning could reduce the population density *of M. alternatu*s and carrying quantity of *B. xylophilus* of each *M. alternatus*. So prescribed burning could control the spread of pine wilt disease and prevent its outbreak. Prescribed burning has an important theoretical and practical significance in pine wilt disease prevention.
Keywords: *Monochamus alternatus, Bursaphelenchus xylophilus, pine wilt disease, fire disturbance, Pinus massoniana*

Introduction

Pine wood nematode disease, also known as pine wilt disease, is a devastating disease of pine. It can damage more than 50 species of pinus and a few non-pinus species and create huge economic losses (Yang et al., 2003; Gu et al., 2005; Pan, 2011; Lee et al., 2013). *M. alternatus* is the main vector of *B. xylophilus*. Nematodes invade healthy pine trees by feeding of adult beetles and by laying eggs of female beetles. Once the pine tree was infected, it died in a short time (Pajares et al., 2010; Teale et al., 2011; Chen et al., 2012; Shi et al., 2019). Studies had shown that *B. xylophilus* moves to the surface of *M. alternatus* before they fly out of the diseased trees, then it enters the body of *M. alternatus* through the ventral valve (Aikawa, 2008; Chen and Tu, 2012). Therefore, it is very important to control the transmission of pine wood nematode disease.

At present, physical, chemical and biological methods, such as trapping device, black light, wood trapping and killing were mostly used to control the population density of *M. alternatus* and *B. xylophilus* (Sanchez-Husillos et al., 2015; Zhu et al., 2017). Contact stomach toxicity, internal absorption, and agents with strong permeability, such as

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thiophosphorus thiaziamprid, chloramine, phosphorescent milk, etc. were generally used in chemical control like artificial and aircraft spray or trunk injection agent (Zhang et al., 2010; Zhan, 2014).

Biological control was dominated by the release of *Metarhizium anisoplia* and *Beauveria bassiana* as well as natural enemies (such as *Dastarcus helophoroides* and *Sclerodermus* SPP.) (Liu et al., 2007; Yang et al., 2013; Luo et al., 2015). Although scholars had done a lot of research and made great progress in the control of *M. alternatus* and pine wilt disease, there were some limitations in various control theories and technologies. It was difficult to popularize physical control in large areas. Chemical control caused secondary pollution and had a high cost. Biological control was less effective (Chou, 2015). Therefore, in order to prevent the occurrence and spread of pine wilt disease, exploring effective control technologies is necessary. This experiment attempted to control the population density of the vector insects of pine wilt disease by prescribed burning.

Prescribed burning refers to using low-intensity fire to remove undergrowth fuel at certain ecological conditions by planned and purposeful artificial control. It is a technique that achieves certain desired results for one or more goals, such as preventing forest fire, promoting natural regeneration, improving wildlife feed sources and reducing forest pests and diseases. Prescribed burning is an important measure for the healthy management of plantation, which is to take advantage of forest fire and reduce the source of diseases and pests in fuels and dead leaves.

Studies in home and abroad had shown that prescribed burning can reduce large-scale outbreaks of insect pests, and it is now used as a strategy to prevent the occurrence of trunk borer pests (Cui and Tian, 2010). Wang found that it was suitable for *Dendrolimus superans* and *Clostera anastomosis* prevention by low intensity prescribed burning (Wang, 2005). The population density of *D. superans* decreased by 1.793 times in prescribed burning plot compared to control plot, and the rate of trees infected by *C. anastomosis* decreased by 78.56 percentage. Cha et al. (2019) researched the control of *Mycosphaerellalaric-leptolepis* Ito et al. by prescribed burning. Prescribed burning reduced the fallen leaves which were infected by diseases. It can achieve the purpose of reducing and controlling the disease, and the effect of prevention was obvious, the cost of prevention was significantly reduced. Futai (2013) used the technology of prescribed burning to control grey spot, poplar leaf rust and poplar rot, and the average control effect were 76.9%, 83.7% and 79.02, respectively. It showed that the effect was remarkable, and the cost of prescribed burning was 20 times lower than that of chemical control. Han et al. (2003) used prescribed burning to prevent forest diseases, pests and mice in Xiaoxing'an Mountains. The results showed that prescribed burning had a good effect on the pest and leaf disease.

More and more attention had been paid to the research on the effects of prescribed burning on forest pests and diseases. It is well known that high intensity fire wreaks forest ecosystems, while low-intensity fire reduces litter, the combustibility of stand and the source of diseases and insects in the forest. Low-intensity fire promotes the sustainable development of forest ecosystems.

In this study, the technology of low-intensity prescribed burning was set up in the experimental stands. In order to find a new prevention and control technology, we want to study the effect of fire disturbance on the population density of *M. alternatus* and pine wilt disease. It has great significance in the theoretical and technical innovation of pine wilt disease prevention and control.

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Materials and methods

Study area

The experiment plot was located in Ningxiang County, Hunan province, with a latitude of 27°55′N to 28°06′N and a longitude of 112°39′E to 112°47′E. It belongs to Hilly landform, the terrain is higher in the northwest and southeast, while there is an alluvial plain in the middle (Huang, 2014). It is characterized by mainland monsoon climate. The alternation of warm and cold air are more obvious, with four distinct seasons. The average annual temperature and the average annual precipitation are about 16.6℃ and 1384.2 mm, respectively. The area is dominated by subtropical evergreen broadleaved forests with a forested cover of 42%. The coniferous forests consist of *Chinese fir* (*Cunninghamia lanceolata* [Lamb.]) and *Pinus massoniana* primarily, and broadleaved forests are dominated by *Phyllostachys heterocycla*, *Schima superba* and *Liquidambar formosana*. These were grouped into pure coniferous, pure broadleaved, and mixed forests. The economic forests are *Citrus reticulata*, *Camellia oleifera*, *Vernicia fordii* and *Sapium sebiferum*.

Experimental design

The trapper consists of an umbrella cover, a circular funnel, a cross baffle and a bug barrel. Attractants (APF-Ⅰ) and the trapper were produced by Fujian Chenkang agricultural and forestry technology co. LTD. In addition, various instruments and materials, such as anatomic tools, gauze, distilled water, hydro extractor, dropper, microscope slide, cover slide and microscope were used in the experiment.

All the plots were located in the middle slope, with a latitude of 28°1′N and a longitude of 112°44′E. The average height of the stand was 7 to 8.5 m, the average diameter at breast height of the stand was 18 to 24 cm, and the average age was 28 to 30 years and the canopy closure was 0.8. There were about 55 *Pinus massoniana* trees in each plot. Three kinds of stands, such as healthy forest (Ⅰ), infected forest without prevention (Ⅱ) and infected forest with prevention (Ⅲ), were selected in the experimental area on 11th April , 2019. Infected forest without prevention meant that there were infected trees in the stands but the infected trees were not removed. Infected forest with prevention meant that there were infected trees, which were cut down and burned. Medicine was applied on the cut hill.

Six plots were set in each experimental stand (*Figure S1*). The control plot and prescribed burning plot were repeated 3 times, so 18 plots were set up and individually numbered in *Figure 1*.

The distance between every two experimental stands was more than a 1000 m, and the distance between each group of plots was at least 50 m. The size of each plot was 30 m×30 m. The stand was a residual stand of *Pinus Massoniana* plantation after being destroyed. There were few trees in understory and shrub layer. A few other broad-leaved tree species were scattered in forest gaps.

The dead tree of pine wilt disease in infected forest was diagnosed with forest symptom. The needles of the whole crown become reddish brown, hanging upside down on the branches, and it was like fire from distant view. Then, we numbered all the dead trees. The prescribed burning plots (Ⅰ-2, Ⅱ-2 and Ⅲ-2) were cleaned up (*Figure S2*). In order to prevent the formation of forest fire weeds, shrubs and branches were cut down (branches of the trunk less than 2.5 m) and a control line with at least 10 m width was set around the prescribed burning plot.

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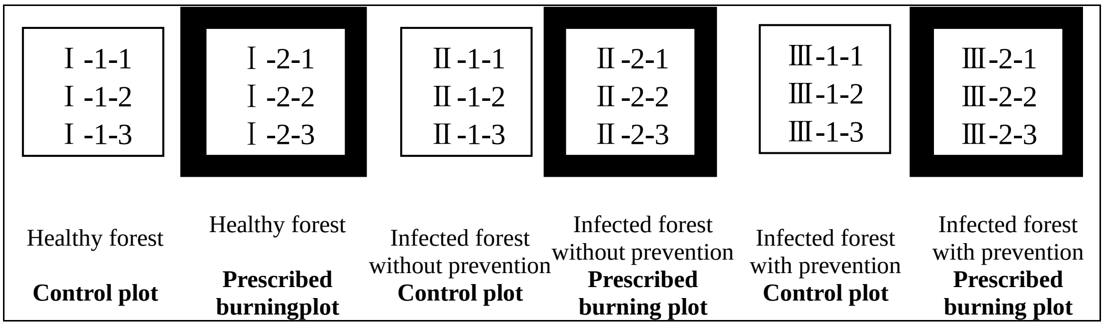

Figure 1. *Experimental design. Note: The black frame was the control line of prescribed burning plot, all of which were 10 m width*

Trappers set up and adult insect observation

In May, 2019, we began trap experiments in 18 plots. The trappers were numbered and hung in the middle of each plot (*Figure S3*). In order to maintain air circulation, weeds, shrubs and branches (which were less than 2.5 m) around the trapper were removed. The trapper was suspended from side branch of the pine tree and the bottom of the trapper was 1.5 m above the ground. To avoid collecting water of the insect bucket, 8 drain holes about 2 mm were drilled at the bottom of the insect bucket. The number of adult beetles of *M. alternatus* were recorded every 10 days during the trapping period, it included the number of females, males and the total *M. alternatus* (*Figure S4*). Numbers of *M. alternatus* which carried *B. xylophilus* were recorded. The number of *B. xylophilus* were recorded, it included the average number of *B. xylophilus* carried by females, males and the total. The attractant was replaced every 20 days.

Experiment of prescribed burning

When it was sunny continuously for five days, and there was no strong wind, we carried out prescribed burning in the non-control plots during the emergence period of *M. alternatus*. Prescribed burning was carried out in the early morning or late afternoon on the 28th June, 2019 (*Figure S5*). The wind direction and wind speed were measured before ignition, then the portable oil-drop igniter was used to light the fire every 1 m in the plot. The fire line was perpendicular to the wind direction, and the combustion effect was checked after the fire.

Identifying the female and male M. alternatus, B. xylophilus isolation

(1) Male and female *M. alternatus* were identified based on the tentacles.

(2) Isolation procedure of *B. xylophilus*: According to the principle of Baermann funnel method, an experiment was designed to isolate nematodes (Cai and Jiang, 2003). We cut up the adult beetle and wrapped debris with 2 layers of gauze, then placed it in a 10 ml centrifuge tube to which 7 ml distilled water was added. The gauze covering the fragments of *M. alternatus* was completely suspended in water (*Figure 2*). It was put aside for 24 hours, then the gauze was removed from the centrifuge tube by forceps. We placed the centrifuge tube in a centrifuge. The supernatant was centrifuged at 3000 R/Min for 3 min, then the supernatant was discarded.

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Figure 2. *Bursaphelenchus xylophilus isolation. Note: Laboratory site: Laboratory of Forestry College in Central South University of Forestry and Technology (112º59'35"E, 28º8'15"N)*

(3) Counting *B. xylophilus*: The supernatant was stabillized to 1.5 ml with distilled water and fully shaken to absorb 0.1 ml with a pipette, the liquid was put on a microscope slide, then it was covered by a cover slide. *B. xylophilus* was observed under microscope and counted, the procedure was repeated 5 times. The number of *B. xylophilus* carried by each *M. alternatus* were calculated.

$$A = b \times 15 \tag{Eq.1}$$

where, A is the numbers of B. xylophilus (per adult) and b is the average of five observations (per 0.1 ml).

M. alternatus were collected every 10 days from the 23rd May to the 18th July, 2019. *B. xylophilus* carried by *M. alternatus* were isolated and counted according to the above methods.

Statistical analysis

Professional software of Excel 2010 and SPSS 23 were used for data processing. One-way ANOVA of SPSS was used for variance analysis and Duncan's new complex range test was used to compare the difference levels between each treatment.

Results and discussion

Quantity of M. alternatus at different time nodes

The quantity of *M. alternatus* adults in 18 plots before and after the fire were analyzed. The quantity of *M. alternatus* in all kinds of plots before and after the fire disturbance were few, so *M. alternatus* in three kinds of plots were combined for statistical analysis. As time went on after the fire disturbance, *M. alternatus* decreased significantly. It showed that the quantity of *M. alternatus* in 20 days after the fire were lower than that of 10 days after the fire, and the quantity of *M. alternatus* in 10 days after the fire were lower than that of before the fire. There were significant differences of *M. alternatus* in stand at different time nodes (P<0.05) (*Figure 3a*).

The results indicated that the quantity of female *M. alternatus* in three kinds of fire disturbance plots were reduced by 88.24%, 75.00% and 94.74%, respectively, while the male reduced by 77.78%, 81.82% and 88.89% before and after the fire. The difference in numbers between males and females were unanimous. The quantity of female *M. alternatus* were larger than male at different time nodes, but there were no significant differences (*Figure 3b*).

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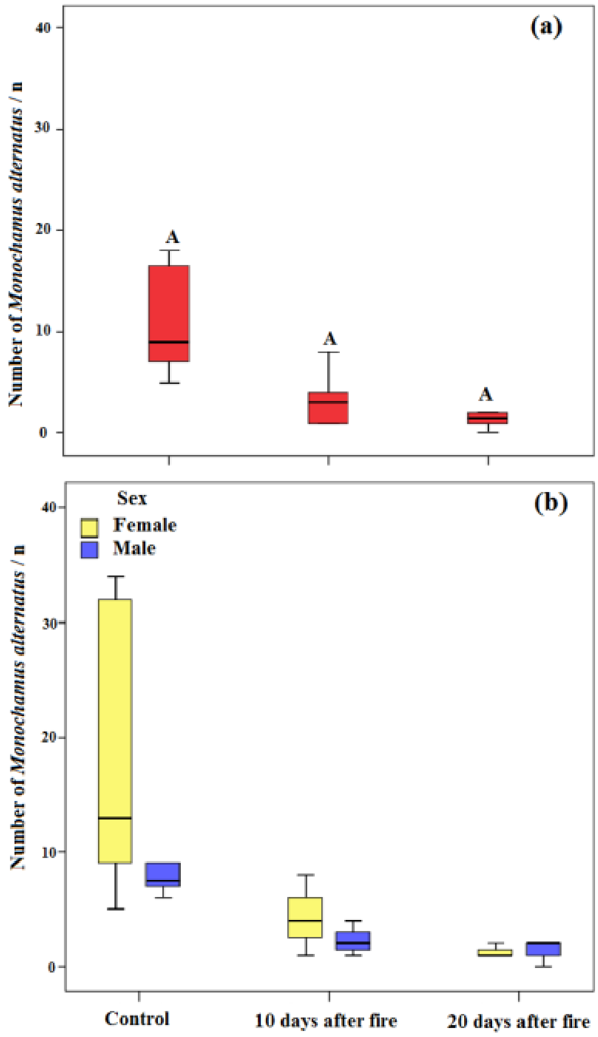

Figure 3. *Changing trend of quantity of M. alternatus at different time nodes. Note: The same letters represent significant differences (One-Way ANOVA of variance, Fish LSD of test, p<0.05). The notes in following figures were the same as in Figure 3*

B. xylophilus at different time nodes in healthy forest

In stands (I), the quantity of *B. xylophilus* at different time nodes decreased with the passage of time after the fire (10 days and 20 days) (*Figure 4a*). Within 10 to 20 days after the fire, *B. xylophilus* changed significantly (*P*=0.03). However, there were no significant differences of *B. xylophilus*in 10 days after the fire comparing with before and 20 days after the fire. *B. xylophilus* carried by female and male *M. alternatus* decreased with the passage of time after the fire. *B. xylophilus* carried by male *M. alternatus* changed significantly before and after the fire (*P*<0.05), but there were no significant differences between 10 days after the fire and 20 days after the fire. *B. xylophilus* carried by female *M. alternatus* showed significant differences between 20 days after the fire (6.33±1.97) and before the fire (1209.14±58.07) (*P*=0.01), and *B. xylophilus* 20 days after the fire were significantly lower than 10 days after the fire (1031.14±50.75, *P*=0.04) (*Figure 4b*).

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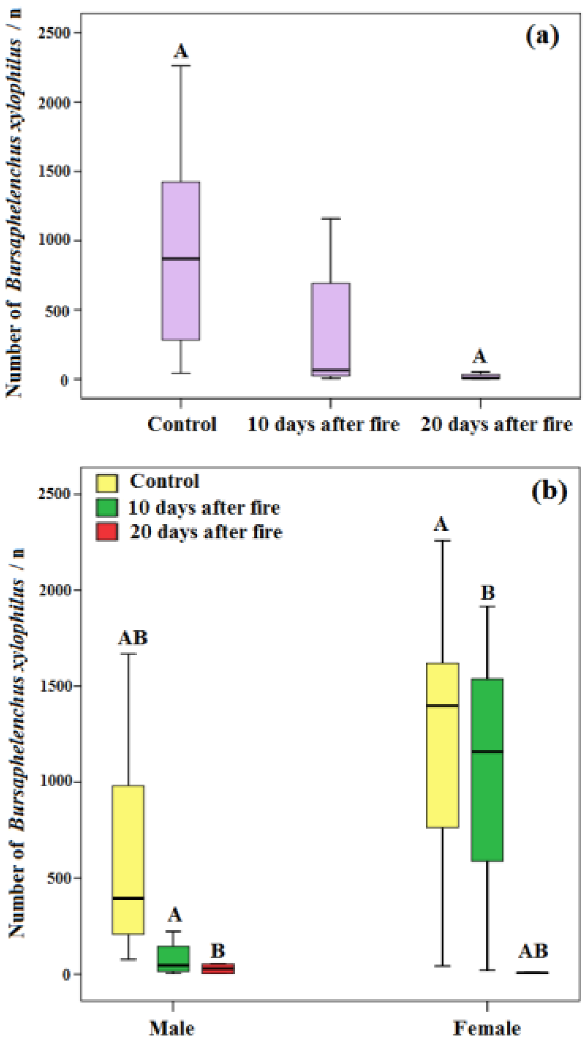

Figure 4. *Significance of B. xylophilus at different time nodes in healthy forest*

B. xylophilus at different time nodes in infected forest without prevention

In stands (Ⅱ), the quantity of *B. xylophilus* 10 days after the fire were significantly higher than in other time nodes (*P*<0.05), while there was no significant difference between 20 days after and before the fire (*Figure 5a*). *B. xylophilus* carried by female and male *M. alternatus* had the same change trend at different time nodes. *B. xylophilus* carried by male *M. alternatus* were significantly different at different time nodes. 10 days after the fire (316.00±74.17) the number of *B. xylophilus* were significantly larger than that before the fire (119.23±6.77), and 20 days after the fire (54.50±16.53) *B. xylophilus* decreased significantly. *B. xylophilus* carried by female *M. alternatus* were significantly different at different time nodes consistently with male *M. alternatus*. Compared with before the fire, *B. xylophilus* increased significantly 10 days after the fire (*P*=0.007) and decreased significantly 20 days after the fire (*P*=0.003). Within 10 to 20 days after the fire, *B. xylophilus* carried by female and male *M. alternatus* in prescribed burning plots were significantly lower than before the fire. The results indicated that prescribed burning could control the spread of pine wilt disease, but the effects do not appear immediately after the fire (*Figure 5b*).

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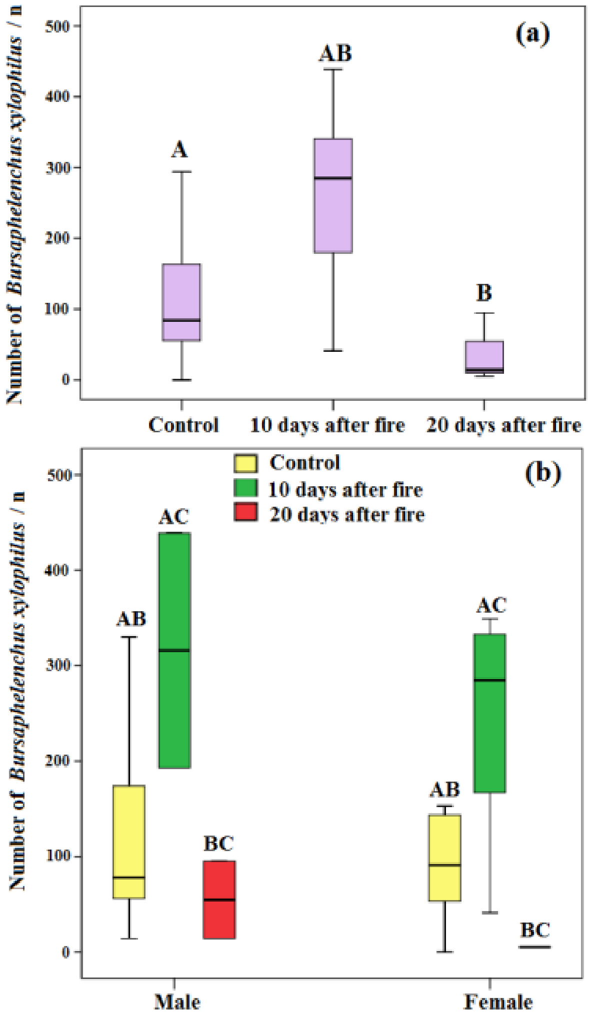

Figure 5. *Significance of B. xylophilus at different time nodes in infected forest without prevention*

B. xylophilus at different time nodes in infected forest with prevention

In stands (Ⅲ), changes of *B. xylophilus* were basically the same as in other stands (Ⅰ) (*Figure 6a*). *B. xylophilus* decreased with the passage of time after the fire in both female and male *M. alternatus*. *B. xylophilus* carried by male *M. alternatus* were significantly lower 20 days after the fire than 10 days after (P=0.012), and before the fire (P=0.000), while *B. xylophilus* did not decrease significantly 10 days after the fire. *B. xylophilus* carried by female *M. alternatus* before the fire were significantly larger than that of 10 days after the fire (P=0.031), and *B. xylophilus* carried by female *M. alternatus* 10 days after the fire were significantly larger than that of 20 days after the fire (P=0.000). There were significant differences among different time nodes (P<0.05) (*Figure 6b*).

Effectiveness of B. xylophilus prevention by prescribed burning

In three kinds of stand, within 10 to 20 days after the fire, *B. xylophilus* carried by female and male *M. alternatus* reduced significantly (carried by female: P=0.01; carried

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by male: *P*=0.022). The average quantity of *B. xylophilus* carried by female and male *M. alternatus* dropped from 1135 to 6 and from 397 to 35, respectively. It showed that prescribed burning could reduce the population density *of M. alternatu*s and quantity of *B. xylophilus* carried by *M. alternatus*. So it is beneficial to use prescribed burning to control the spread of pine wilt disease and prevent its outbreak (*Table 1*). This was consistent with the findings of Wang et al. (2019) that the amount of *B. xylophilus* carried by female *M. alternatus* were about 1.29 times of male adults.

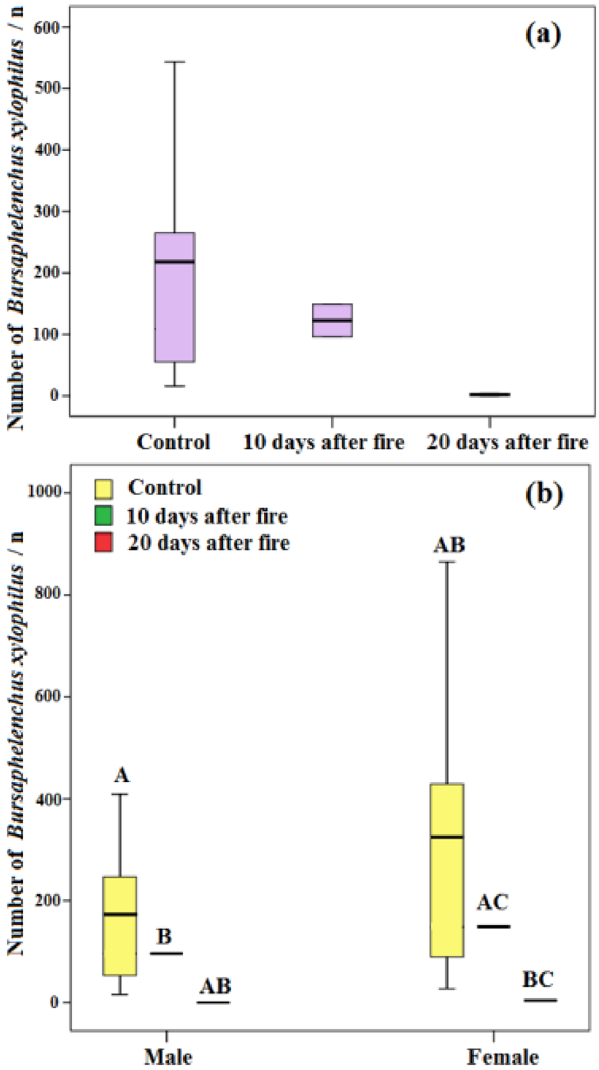

Figure 6. *Significance of B. xylophilus at different time nodes in infected forest with prevention*

Comparison of prevention effectiveness by prescribed burning

The mean number of *M. alternatus* in three kinds of stand were analyzed before and after the fire, the results showed that the quantity of *M. alternatus* in three kinds of fire disturbance plots were reduced, but only stands Ⅱ-2 and Ⅲ-2 reduced significantly (Ⅱ-2: *P*=0.012, Ⅲ-2: *P*=0.026). So it indicated that the prevention efficacy of fire disturbance in plots Ⅱ and Ⅲ were significantly better than in plot Ⅰ, and the prevention efficacy of Ⅱ-2 was the best (*Figure 7*). Whether it was the high

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temperature that killed the pupa in the xylem of *Pinus massoniana* or that killed the emergence of adult beetle, or other mechanisms and the comprehensive effect of various factors need further research.

Table 1. *B. xylophilus carried by female and male M. alternatus before and after the fire*

Plots serial	Trapping time	Female *M.alternatus* (n)	*B.xylophilus* carried by female *M. alternatus* (n)	Male *M.alternatus* (n)	*B.xylophilus* carried by male *M.alternatus* (n)
Ⅰ-2	Before the fire	20	1073	9	420
Ⅱ-2		28	740	8	556
Ⅲ-2		19	1593	8	216
Ⅰ-2	10 ~20 days after the fire	2	7	2	29
Ⅱ-2		1	7	2	55
Ⅲ-2		1	4	2	20

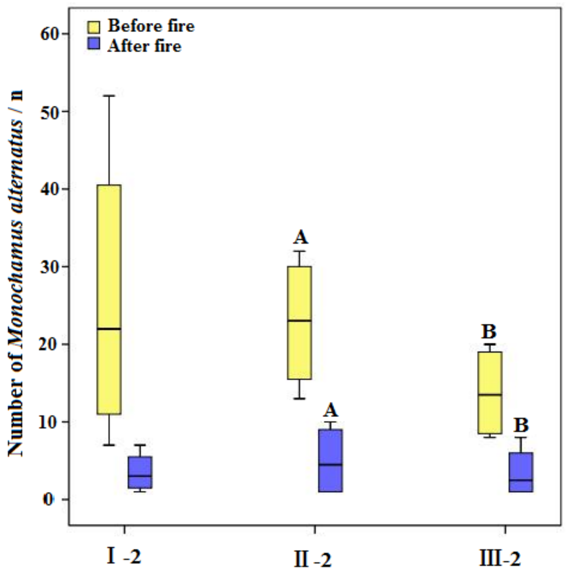

Figure 7. *Significance of M. alternatus in different plots*

Conclusion

In this study, there were no significant changes of *B. xylophilus* carried by female and male *M. alternatus* within 10 days after prescribed burning. However, within 10 to 20 days after prescribed burning, *B. xylophilus* carried by *M. alternatus* decreased significantly. This may be due to that *M.alternatus* in the upper part of the tree were not affected by fire and smoke, so neither *B. xylophilus* carried by *M. alternatus* were affected by fire. However, *M. alternatus* adult beetle, chrysalis and *B. xylophilus* stayed on the lower part of the trunk, thus these were inevitably affected by fire. This explains the sharp drop in the number of *M. alternatus* after prescribed burning, while the reduction in the number of *B. xylophilus* was delayed. In this study, *B. xylophilus* carried by female *M. alternatus* were significantly higher than that by male. The results showed that prescribed burning significantly reduced the population density of *M. alternatus* in *pinus massoniana* forest.

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The control efficacy of prescribed burning in plot (Ⅲ-2) was not as good as in plot (Ⅱ-2), the main reason is as follows: Firstly, it may be due to the occurrence of pine wilt disease. The infected trees had a strong attraction to *M. alternatus*, and the early stage of dead trees of pine wilt disease is often distributed in small groups, so *M. alternatus* are often distributed intensively (Parker et al., 2006). Secondly, the dead trees in the plot (Ⅲ-2) had been cut down and burned on the spot, so the distribution of *M. alternatus* scattered on the plot. The process of burning dead trees released heat and smoke, so *M. alternatus* spread to the surrounding.

It was an innovative research to control pine wilt disease by prescribed burning. The control efficacy is obvious, the cost of control technology is significantly reduced, and the method is simple and easy to operate. It can not only prevent the outbreak of pine wilt disease, but also promotes the transformation of ground litter, reduces combustion of stand and enhances soil fertility. In addition, prescribed burning has low cost, which makes up for the deficiency of existing prevention and control technologies. The technology has broad prospects in application. However, the question of whether high temperature killed the pupa in the xylem of *Pinus massoniana* or it prevented the emergence of adult beetle needs further research along with other questions (such as mechanism and the comprehensive effect of various factors).

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APPENDIX

SUPPORTING INFORMATION

Figure S1. *Pictures of plot design*

Figure S2. *Pictures of cutting down the weeds, shrubs and branches*

Figure S3. *Pictures of trap experiments*

Figure S4. *Pictures of adult insect observation*

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Figure S5. *Pictures of experiment of prescribed burning*

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