

RESEARCH ARTICLE

Population dynamics of *Bursaphelenchus xylophilus* in *Pinaceae* hosts with varying resistance

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Bursaphelenchus xylophilus is the main pathogen of pinewood nematodiasis. Its dynamic change of the population is the key factor influencing the strength of host's disease. This study clarified the reproductive strategies and the population dynamics of *Bursaphelenchus xylophilus* in host trees of different resistance and established the theoretical basis of the early diagnosis of pinewood nematodiasis and the breeding of resistant cultivars. Artificial inoculation tests were performed on three species of hosts of varying degrees of resistance including *Pinus massoniana*, *Larix gmelinii* var. *principis-rupprechtii*, and *Pinus taeda*. The *B. xylophilus* strain BXM6 was introduced into the hosts by the bark inoculation method with 2,000 nematodes per tree. Nematodes were isolated using Bellman funnel method to quantitatively study change in population density and sex ratio of the nematodes inside needles and stems. Samples were taken at 3, 7, 10, 15, 20, 25, 33, and 40 days after inoculation. The sex ratio of the nematode population showed a tendency to increase followed by a decrease in needles and stems of all the host species irrespective of their resistance. The highest sex ratio recorded was for the least resistant *P. massoniana* followed by the moderately resistant *L. gmelinii* var. *principis-rupprechtii* and the lowest for the highly resistant *P. taeda* ($P < 0.05$). In the same manner, the density of the nematodes in needles and stems increased and then decreased with population growth rate being positively correlated with population density. *P. massoniana* showed the highest growth rate and the highest population density, while *P. taeda* showed the lowest growth rate and population density, and *L. gmelinii* var. *principis-rupprechtii* was in the middle ($P < 0.05$). The results indicated that sex ratio of nematode populations and population dynamics were greatly affected by host resistance. Greater host resistance was associated with a lower sex ratio and lesser populations and led to a decline in the diffusion rate of the populations, while its pathogenicity to the host also weakened accordingly.

Keywords: *Bursaphelenchus xylophilus*; host resistance; sex ratio; population quantity.

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Introduction

The primary pathogen of pinewood nematodiasis, *Bursaphelenchus xylophilus*, primarily infects species of the genus *Pinus*, but can also infect a limited number of non-pine

coniferous species [1-3]. Pinewood nematodiasis is known to occur in Europe [4], America [5], Asia [6], and Africa [7]. 52 countries around the world have declared it as a quarantine pest. *B. xylophilus* can infect 51 species of *Pinus* and 16 non-pine species under natural conditions. Its

pathogenicity to different species shows significant differences [8]. The spread of pinewood nematodiasis has increased since its initial appearance in Nanjing, Jiangsu, China in 1982, and by the beginning of 2025, it had reached 17 provinces and 620 counties throughout China [9]. Some naturally susceptible species are *Pinus thunbergii*, *Pinus massoniana*, *Pinus elliottii*, *Larix gmelinii* var. *principis-rupprechtii*, *Pinus koraiensis*, *Pinus taeda*, *Pinus sylvestris* var. *mongolica*, *Pinus tabuliformis*, and others.

Upon entering a pine tree, *B. xylophilus*' high reproductive potential and its rapid population growth are among the main factors contributing to the outbreaks of pinewood nematodiasis [10]. Sex ratio of the nematode is an important factor which determines the mating efficiency, reproductive rate, and population dynamics [11, 12]. *B. xylophilus* has been shown to be able to produce a generation in 3 days when the temperature is 30°C, and the time of reproduction is shortened by higher temperature [8]. Nematodes were found in the trunk of *P. massoniana* 4-year-old trees just 3 days after inoculation and were completely distributed throughout the trees 5 days after inoculation. Needles showed a loss of green color on day 10 and density of the population showed exponential growth. Branches and needles were completely withered and red brown in color by about day 35 [13]. The reproductive stage nematodes multiply and move through the resin ducts and vascular tissues in healthy pines leading to tree death in 40 - 50 days in optimal conditions [14]. *B. xylophilus* is thought to infect trees in natural settings *via* wounds created by the feeding of vector insects on young branches and bark [15, 16]. Nematodes have also been shown to be transmitted by pine needles. Inoculation of three-year-old *P. thunbergii* needles led to the detection of nematodes 24 hours after. Large numbers of nematodes were isolated from needles after the withering of the host plants [17]. The more rapidly *B. xylophilus* reproduces in a host, the faster the population spreads. Host resistance and environment will

influence this spread rate [18]. Many investigations have shown the presence of pinewood nematodiasis resistant species of trees. Differences in resistance have been found among different pine species [19-21]. *P. taeda* has shown strong resistance to pinewood nematodiasis in forestry operations and in scientific trials with no mass pine wilts or deaths reported. In the experiments, based on the disease symptoms, the disease of *L. gmelinii* var. *principis-rupprechtii* appeared 6 days later than that of *P. massoniana* in four-month-old plants. The average wilting rates of *P. massoniana* and *L. gmelinii* var. *principis-rupprechtii* were 89.2% and 53.3%, respectively, by day 25 post-inoculation. In addition, the nematode population in *P. massoniana* was about double that in *L. gmelinii* var. *principis-rupprechtii*, suggesting that *L. gmelinii* var. *principis-rupprechtii* is more resistant [22].

The existing approaches to controlling pinewood nematodiasis include chemical, biological, and physical methods, but these methods are effective yet costly and harmful to the environment [23, 24]. There is little work on breeding resistant cultivars. Moreover, few studies have investigated the dynamics and sex ratio of *B. xylophilus* in hosts of different resistance, especially in needles. The relation of nematode population dynamics, sex ratio, and host resistance is not understood, and this is an important challenge for successful management of pinewood nematodiasis. This study explored the reproductive strategies and population dynamics of *B. xylophilus* by using the bark inoculation method [25] in three host species with different resistance properties to gain theoretical insights in early diagnosis of pinewood nematodiasis and develop resistant cultivars.

Materials and methods

Experimental site materials

The experiment was carried out in the nursery of Xinyang Agriculture and Forestry University

(114°12'E, 32°16'N) (Xinyang, Henan, China) with subtropical monsoon humid climate with distinct seasons, plenty of sunlight, and rainfall. The strain of *B. xylophilus* BXM6, which was originally isolated from naturally infected *P. massoniana* in Ma'anshan Forest Farm (117°53' - 118°52'E, 31°24' - 32°02'N) (Ma'anshan, Anhui, China) with the ability to reproduce and be pathogenic, was used for this study. Nematodes were extracted using Baermann funnel method and were maintained long-term on *Botrytis cinerea* cornmeal agar medium (Hopebio Co., Ltd., Qingdao, Shandong, China) [26]. The host plants were three-year-old *P. massoniana*, *P. taeda*, and *L. gmelinii* var. *principis-rupprechtii* obtained from Qingxuan nursery stock Co., Ltd. (Xinyang, Henan, China) and placed in the potting nursery of Xinyang Agriculture and Forestry University.

Nematode inoculation and isolation

The experiment was conducted from July 10 to August 20, 2025. The bark inoculation method was used to inoculate the three host species. The point of inoculation was mid-stem. 200 µL of nematode suspension (approximately 2,000 pine wood nematodes) was inoculated into each tree. Twenty-four (24) trees were inoculated per host species for a total of 72 trees. In addition, nine trees (three of each species) were inoculated with sterile water as controls. The trees were placed under normal nursing conditions after they were inoculated. Sampling was performed on 3, 7, 10, 15, 20, 25, 33, and 40 days after inoculation (DAI). Each sample consisted of three trees of each host species, while control trees were not sampled [27, 28]. Whole seedlings were dug up and thoroughly washed with running water. The stems were sliced in 1 cm slices, and the needles were split into three parts. Stems and needles were treated as two separate lots. Nematodes were extracted using the Baermann funnel method. The nematodes were collected from the stems and the needles of each host species and were put into three Corning 15 mL centrifuge tubes with clear markers of collection parts. After centrifugation at 5,000 rpm for 5 minutes, the supernatant was removed. The

samples were then adjusted to 10 mL and kept at 4°C for further studies [28].

Sex ratio and population count

50 µL of nematode suspension from 10 mL nematode sample was put on a glass slide. Nematodes were differentiated to males and females under an Olympus CZX51 optical microscope (Olympus, Tokyo, Japan) according to the morphology of the reproductive organs. The L_{IV} juveniles and adults having vulvae were counted as females, while that having spicules were counted as males [29]. The reproductive organs and body length of nematodes were used for the identification of the developmental stages. Juveniles in stages L_{II}, L_{III}, and L_{IV} without visible reproductive organs were put under the category of juveniles. The number of eggs was also counted based on their morphology [30]. The numbers of males and females were used to determine sex ratio and the number of adults, while juveniles and eggs were used to estimate the population. Nematodes were counted separately from stems and needles, and 10 counts were taken of each centrifuge tube.

Data analysis

The data were analyzed using SPSS 25.0 (IBM, Armonk, New York, USA). Analysis of variance (ANOVA) was used to determine the significance of the differences in sex ratio and number of individuals in each host species. Post hoc comparisons were made using Duncan's multiple range test at the 0.05 level of significance.

Results and discussion

Changes in the sex ratio of *B. xylophilus* populations in needles

The sex ratio of *B. xylophilus* in the needles of the three host species showed a trend of increasing first and then decreasing with the significant differences among the three species ($P < 0.05$). The sex ratio of needles from *P. massoniana* was the highest on the 10th day after inoculation, reaching 2.12. The largest sex ratio for *L. gmelinii*

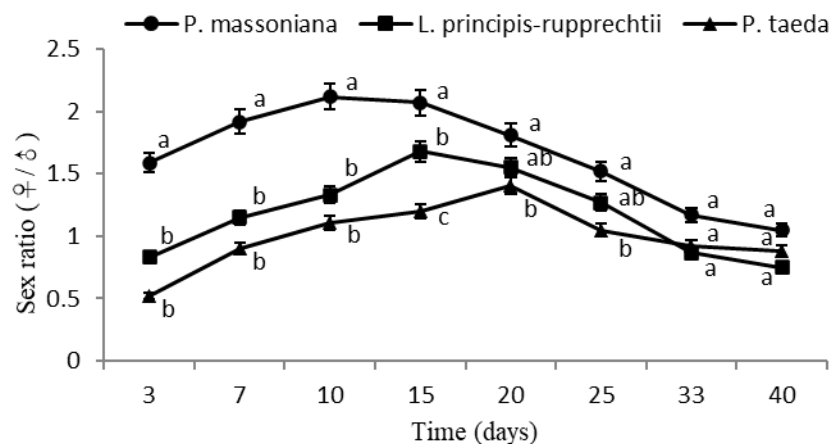


Figure 1. Sex ratio of *B. xylophilus* in the needles of three host species. Different lowercase letters indicated significant differences in the sex ratio of *B. xylophilus* among the needles of the three host species ($P < 0.05$).

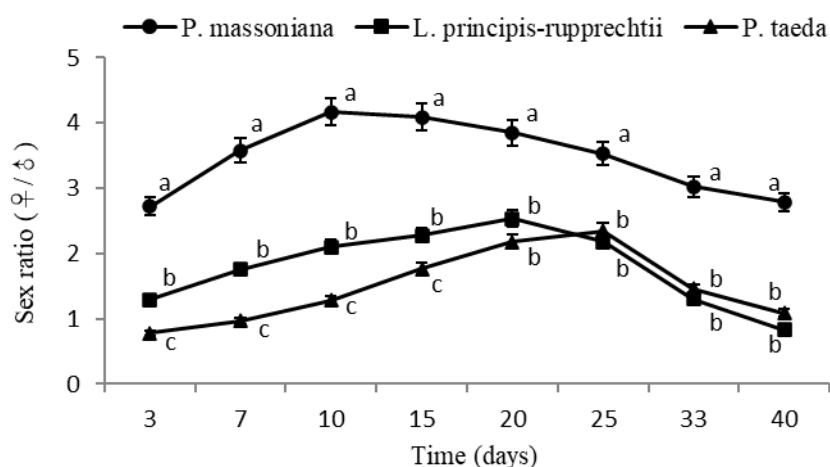


Figure 2. Sex ratio of *B. xylophilus* in the stems of three host species. Different lowercase letters indicated significant differences in the sex ratio of *B. xylophilus* among the stems of the three host species ($P < 0.05$).

var. principis-rupprechtii was 1.68 on the 15th day after the inoculation. For *P. taeda*, the highest sex ratio was 1.41 on the 20th day after inoculation. The sex ratio of *B. xylophilus* in needles of *P. massoniana* was significantly higher than that of *L. gmelinii var. principis-rupprechtii* and *P. taeda* within the first 25 days after inoculation ($P < 0.05$) (Figure 1).

Changes in the sex ratio of *B. xylophilus* populations in stems

The sex ratio of *B. xylophilus* populations in the stems of the three host species also showed an increase and subsequent decrease trend with significant differences among the species ($P <$

0.05). The highest sex ratio of *P. massoniana* in the stems was 4.17 on the 10th day after inoculation, while the maximum sex ratio of *L. gmelinii var. principis-rupprechtii* was 2.53 on the 20th day after inoculation. The highest sex ratio of *P. taeda* was 2.35 on the 25th day after inoculation. Significant differences in sex ratio in stems of *L. gmelinii var. principis-rupprechtii* and *P. taeda* were observed within 15 days after the inoculation ($P < 0.05$). The sex ratio in the stems of *P. massoniana* was significantly higher than that in *L. gmelinii var. principis-rupprechtii* and *P. taeda* during the whole experiment ($P < 0.05$) (Figure 2)

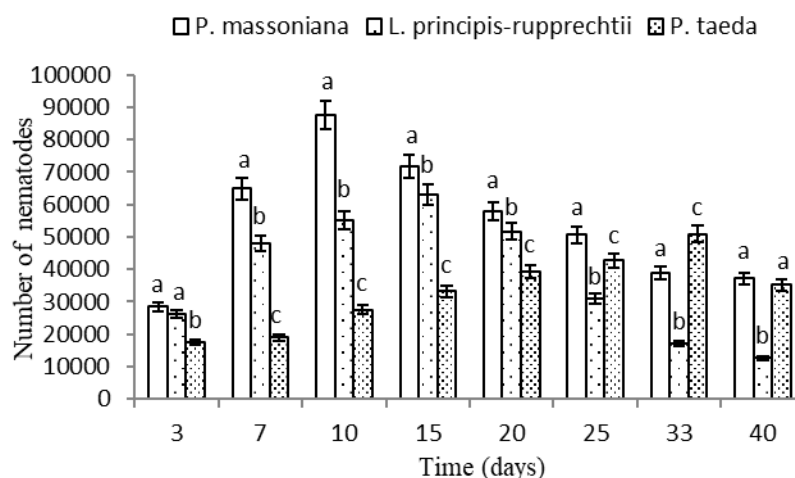


Figure 3. Population density of *B. xylophilus* in the needles of three host species. Different lowercase letters indicated significant differences in the population density of *B. xylophilus* among the needles of the three host species ($P < 0.05$).

Changes in population density of *B. xylophilus* in needles

The population density of *B. xylophilus* in the needles of the three host species showed a pattern of increase and decrease in the density of *B. xylophilus* with significant differences among the species ($P < 0.05$). The highest population density of *P. massoniana* in needles was 87,591 on the 10th day after inoculation. The maximum population density for *L. gmelinii* var. *principis-rupprechtii* was 63,079, which occurred on the 15th day after the inoculation. The day with the highest population density in *P. taeda* was the 33rd day after inoculation with a value of 50,936. The population density in the needles of the three host species was in the order of *P. massoniana* > *L. gmelinii* var. *principis-rupprechtii* > *P. taeda* within the first 20 days post-inoculation ($P < 0.05$) (Figure 3).

Changes in population density of *B. xylophilus* in stems

The population density of *B. xylophilus* in the stems of the three host species also demonstrated first increased and then decreased with significant differences ($P < 0.05$). The highest population density for the stems of *P. massoniana* was 341,909 on the 15th day after inoculation. The highest number of *L. gmelinii* var. *principis-rupprechtii* was 289,073 on the 25th

day after the inoculation. The maximum population of *P. taeda* was 248,740 on the 25th day after inoculation. The population density in the stems of the three host species was arranged in the order of *P. massoniana* > *L. gmelinii* var. *principis-rupprechtii* > *P. taeda* and differed significantly within the first 20 days of post-inoculation ($P < 0.05$) (Figure 4).

Discussion

Pinewood nematodiasis is a complex disease system with *B. xylophilus* being the only pathogen and other factors such as bacteria, fungal food sources, vector insects, host plants, and human intervention playing a role in the disease [31]. It has been shown in previous studies that the greater the reproductive activity of *B. xylophilus* in a pine tree, the quicker the spread and the more the tree is damaged [32]. The reproductive and dispersal rate of the nematode is closely related to the sex ratio of the nematode and host resistance [33]. The results of this study showed that the sex ratio of *B. xylophilus* populations was the highest in the needles and stems of *P. massoniana* and was significantly different from the other species of *L. gmelinii* var. *principis-rupprechtii* and *P. taeda* in the experimental period ($P < 0.05$). The sex-ratio

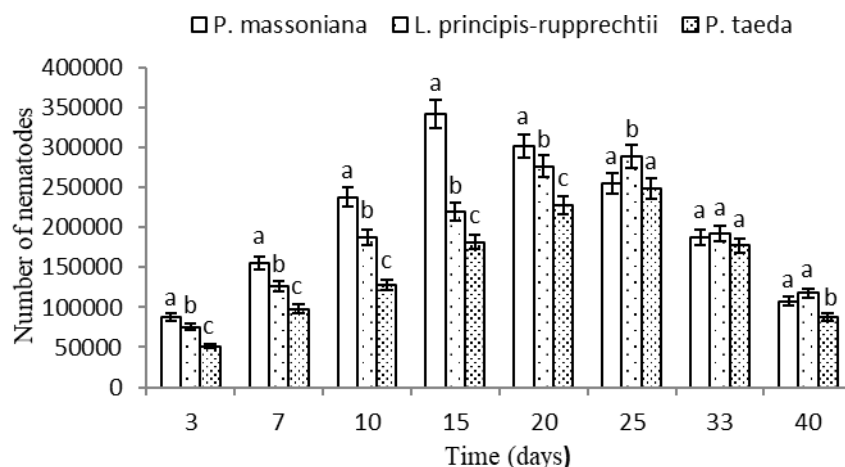


Figure 4. Population density of *B. xylophilus* in the stems of three host species. Different lowercase letters indicated significant differences in the population density of *B. xylophilus* among the stems of the three host species ($P < 0.05$).

of *L. gmelinii* var. *principis-rupprechtii* was significantly higher than that of *P. taeda* in the stems ($P < 0.05$) but did not significantly differ in the needles within the first 15 days after inoculation. The results indicated a greater inhibitory effect on the sex ratio of nematode population under a higher level of host resistance and showed that *B. xylophilus* produced a higher sex ratio in favorable environments and a lower sex ratio in less favorable environments. Further, under unfavorable environmental conditions, *B. xylophilus* populations tended to produce more male offspring, whereas under favorable conditions, they tended to produce more female offspring to sustain population growth [34, 35].

Population density of *B. xylophilus* in needles and stems of the three host species tended to increase and then decrease during the experimental period and was significantly different among the species ($P < 0.05$). The population of *P. massoniana* in the needles increased most rapidly and peaked at the 10th DAI, which was the highest population density of the three species. In *L. gmelinii* var. *principis-rupprechtii* the population densities in needles increased at a slower rate and peaked on the 15th day after inoculation. The slowest growth rate was found in *P. taeda*, which also had the lowest population density on the 33rd DAI ($P < 0.05$). Besides, chlorosis was seen in some needles of *P.*

massoniana as soon as the 8th day after the inoculation. These results indicated that the sex ratio of nematode populations increased faster in the needles of less resistant host trees, which, in turn, supported more rapid nematode population growth. The sex ratio, on the other hand, increased more slowly in *P. taeda* due to its greater resistance, thus delaying the population density peak. Many studies have shown that the greater the sex ratio of *B. xylophilus* populations, the greater the reproductive ability and rate of increase of the population [36, 37]. The number of nematodes per stem was greatest in *P. massoniana*, less in *L. gmelinii* var. *principis-rupprechtii* and the lowest in *P. taeda* within the first 20 days after inoculation ($P < 0.05$). By 25 days after inoculation, the population densities of all three host species started to decrease and level off in the stems. The results suggested that the increase in the sex ratio of the stems was also beneficial for population increase, and this increase was sustained. For *L. gmelinii* var. *principis-rupprechtii* and *P. taeda*, the higher resistance contributed to the decrease in sex ratios and population growth rates, which, in turn, decreased the population densities ($P < 0.05$). In addition, the sex ratio and the numbers of nematodes in the needles of all three host species were found to be less than those in the stems during this experimental period, which might be because the space available within the

needle for nematode growth and reproduction was limited, and the tissue structure was relatively simple. Consequently, density-dependent factors played a greater role in influencing population size.

This study analyzed the changes in sex ratio and population quantity of *B. xylophilus* in host pines with different resistance and found that the population quantity of *B. xylophilus* was affected by the sex ratio in host pines. On hosts with higher resistance and a lower sex ratio, the population size of *B. xylophilus* also declined correspondingly. The results could serve as a scientific basis for the early diagnosis of pinewood nematodiasis and selection of resistant cultivars. However, this study was carried out on artificially inoculated potted seedlings, which had some limitations. Future studies should focus on the dynamics of the relevant indicators under natural infection conditions.

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