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## **Inoculation Effects of *Pinus thunbergii* with *Bursaphelenchus xylophilus* and two strains of *Bacillus firmus***

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### **ABSTRACT**

To clarify the role of bacterium in the pathogenesis of pine wilt disease, 3 years old Japanese black pine (*Pinus thunbergii*) were inoculated with disinfected *Bursaphelenchus xylophilus* (Bx), bacterium isolate GD1 of *Bacillus firmus* (isolated from the body of Bx), GD2 of *B. firmus* (isolated from the healthy *P. massoniana*) and the mixture of the nematode and bacterium. The results showed that the pine seedlings were diseased when inoculated with the disinfected Bx and the mixture of Bx and bacterium, while not diseased when inoculated with *B. firmus* singly. Disease development of the pine seedlings was slower after inoculated with Bx singly than with the mixture of Bx and bacterium. The disease of pine seedlings was heavier when the inoculation concentration of bacterium was higher. After inoculated with Bx singly and the mixture of Bx and bacterium, the pith of pine seedlings browned, the process of pith browning was from lower part to upper part of the inoculated main stem, while the pith of pine seedlings was normal after inoculated with *B. firmus* singly and the control. At early stage after inoculation with the mixture of Bx and *B. firmus*, the number of bacterium detected in the pine seedlings was larger. Therefore, it was concluded that the two bacterium strains enhanced the disease development of pine wilt disease.

**Key words:** *Pinus thunbergii*; *Bursaphelenchus xylophilus*; Bacterium; *Bacillus firmus*

### **INTRODUCTION**

Up to now, the pathogenic mechanism of pine wilt disease keeps obscure. The role of bacterium in pathogenesis of the disease is still unclear (Oku *et al* 1980; Kawazu & Kaneko 1997; Zhao & Guo 2004; Zhu *et al* 2012).

## MATERIALS AND METHODS

3 years old Japanese black pine (*P. thunbergii*) were inoculated with disinfected *Bursaphelenchus xylophilus* (Bx), bacterium isolate GD1 of *Bacillus firmus* (isolated from the body of Bx), GD2 of *B. firmus* (isolated from the healthy *P. massoniana*) and the mixture of the nematode and different concentration of bacterium by bark inoculation method. The inoculation number of Bx and bacterium was 3500,  $2.3 \times 10^5$ CFU,  $2.3 \times 10^6$ CFU and  $2.3 \times 10^7$ CFU per seedling. At 5 days after inoculation, the number of bacterium in the inoculation main stem 3-4 cm above inoculation point was detected.

## DISCUSSION

This inoculation experiment was conducted on 3-year-old Japanese black pine seedlings, its results were similar to that on 1-2 years old excised branch of masson pine (*P. massoniana*) (Tan 2001). The bacterium strain GD2 was isolated from the healthy *P. massoniana*, it belongs to pine endophytic bacterium. The research on the relationship between pine endophytic bacterium and pine wilt disease is being carried out.

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