Interspecific hybridization between *Bursaphelenchus xylophilus* and *Bursaphelenchus mucronatus*

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ABSTRACT:

Pine wood nematode (*Bursaphelenchus xylophilus*) is the pathogen of pine wilt disease that causes pine wilt or death. *Bursaphelenchus mucronatus*, belonging to same genus with *B. xylophilus,* was previously considered as no-virulence or weak pathogenicity. Because *B. mucronatus* exists widely in the pine forests of Eurasia, there has been a lot of interest in the hybridization between B. *mucronatus* and *B. xylophilus* and many investigations could be found in the literature. However, the investigations were inconclusive and many issues remained unresolved and unaddressed. For example, the enhance pathogenicity of *B. mucronatu* and the indistinctive classification characteristic of the *Bursaphelenchus mucronatus* and *B. xylophilus* and so on. Gaining new insights on the issues will contribute not only to *Bursaphelenchus* classification, but also to the quarantine and control of the pine wilt disease.

We conducted indoor hybridization using the nematode isolates of *B. xylophilus* from China, Japan and the nematode isolates of *B. mucronatus* from China, Japan and France. Our objectives were to examine the two species in mating ability, hybrid offspring survival and fecundity. The study results would provide experimental evidences on the hybridization of the two species and insights on whether the two species could be merged. Our study has important implications for the classification of genus *Bursaphelenchus* and pine disease quarantine and control.

Male and female adults of the isolates of *B. xylophilus* and *B.mucronatus*, were orthogonal or reverse crossed, with the intraspecific self mated and single female adult cultured as control, cultivated 7 days to observe the F1 generation. All the combinations were able to cross and had the ability to generate F1 progeny, although the offspring counts varied from tube to tube in a range of 17- 44. Compared with intraspecific self mating, the hybrid of *B. xylophilus* and *B.mucronatus* produced a smaller number of F1 generation offspring. In the intraspecific mating, the number of offspring produced each

tube is around 100. In the interspecific mating, however, the number of F1 generation offspring is around 50, only half of what is in the intraspecific mating.

For the research of F2 generation nematodes production , each F1 generation of 10 larva was selected and in *Botrytis cinerea* slope, 25 ℃ cultured 7 day, observed under microscope , and recorded the results. From the results we can see that all the combination has F2 generation produced, just differ in nematodes total quantity, larva rate and nematodes vigor.

In order to understand the reproductive capacity of hybrid offspring, we obtained the hybrid offspring of Chinese combinations (BxZJ×BmCHN). The result indicated that the hybrid Chinese combination could produce up to 22 generations. The average number of larvae increased while the average number of adults decreased with an increasing generation. In addition, nematode mortality increased. But sex ratio was stable from generation to generation.

The backcross outcomes of different cross combinations with their parents were also researched ,and the combination of F1 generation of female adults and their parents were mated. The result indicated that all F1 hybrids could backcross with their parents and produce offspring. Based on these study findings, we propose to merge *B. xylophilus* and *B. mucronatus* into one "species" and each belongs to a ".pathogenic type" under the "species".