

Recent Research on Pine Wilt Disease in Russia

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In early 1990 we initiated a survey in Russian conifer forests to determine if the pine wood nematode (PWN), *Bursaphelenchus xylophilus* occurred in Russia. Later, in 2010-2011 larger, more widely-distributed surveys were conducted for the PWN in conifer forests and in stored lumber in eleven regions of Russia. Based on the results of the surveys *B. xylophilus* has been not found in Russia, however, the closely related nematode species *B. mucronatus* has been found. Specifically in the 3718 samples analyzed *B. mucronatus* was found in 11.5% of the samples in 2010 and 5.6% in 2011. Inoculation experiments done in Russia with *B. mucronatus* isolates showed that sometimes these isolates killed pine (*Pinus sylvestris*) and larch (*Larix olgensis*) seedlings. Also recent research done in China and South Korea showed that PWD of conifers can occur following inoculations done using a complex of pathogenic bacteria and the PWN of *B. xylophilus* that carried them (Zhao *et al.* 2009; Kwon *et al.*, 2010; Wu *et al.* 2013). As well, it has been shown that *B. mucronatus* species carry such bacteria and perhaps some *B. mucronatus* populations can vector such pathogenic bacteria. During our 2010-2012 survey twenty six isolates of the wood-inhabiting nematode *B. mucronatus* were extracted and propagated *in vitro* to determine, using sequencing techniques, the identity of the associated symbiotic bacteria..

Twenty species of bacteria belonging to the families Enterobacteriaceae, Xanthomonadaceae, Pseudomonadaceae, Burkholderiaceae, Rhizobiaceae, Nocardiaceae, Flavobacteriaceae, Bacillaceae, Paenibacillaceae were isolated from the nematodes and identified as belonging to the genera *Achromobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Klebsiella*, *Pseudomonas*, *Rahnella*, *Rhodococcus*, *Stenotrophomonas*, *Pantoea*, *Paenibacillus*, and *Serratia* (Fig 1). The most frequently encountered bacterium belonged to the genus *Pseudomonas* (44%). Five species of this genus were identified: *P. lurida*, *P. brenneri*, *P. geniculata*, *P. fluorescens*, *Pseudomonas* sp. The bacterium *Pseudomonas fluorescens* was isolated from nine *B. mucronatus* isolates from the different regions. Too bacteria-associated species were found on the

dauerlarva stage of *B. mucronatus* nematodes were isolated from beetles identified as *Monochamus urussovi* Fisch. Four species of bacteria including *Pseudomonas fluorescens* species were isolated from the larva stage of a *B. mucronatus* isolate.

According to the results of Chinese researchers, *P. fluorescens* is an essential species of the nematode-bacterial complex that induces PWD in the pine forests of southern China (Zhao, 2008). It is assumed that *B. mucronatus* nematodes and symbiotic bacteria *P. fluorescens* can cause death of some Russian pine forests in areas where the mean air temperature during the summer months exceeds 25°C. The average monthly temperature in the Centre of European Russian in 2010 was 26.4 °C in July and 25.5 °C in August. Widespread death of *Pinus sylvestris* occurred there after 2010. *Bursaphelenchus mucronatus* nematodes and the symbiotic bacterium *Pseudomonas fluorescens* were isolated from some dead trees. Local foresters believe that the death of these trees was caused by drought, but we do not exclude the possibility that that PWD played some role in the death of these trees..

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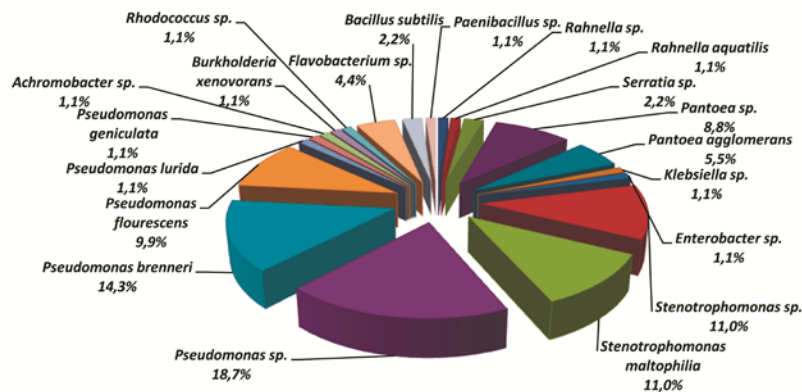


Fig 1. Symbiotic bacteria extracted from *Bursaphelenchus mucronatus* isolates from different regions of Russia