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Inter-specific competition of *Bursaphelenchus xylophilus* with native populations of *B. mucronatus* in pine

Tomalak M, Filipiak A

Institute of Plant Protection – National Research Inst., Węgorza 20, 60-318 Poznań, Poland

Email: M.Tomalak@iorpib.poznan.pl

Both the quarantine pest nematode, *Bursaphelenchus xylophilus* and native to Eurasia, unnharmful *B. mucronatus* are genetically closely related and present similar bionomics. They develop and reproduce in pine, can use the same insect vectors, and with continuous colonization of new localities by *B. xylophilus*, the overlap of their geographic distribution continues to increase. The laboratory and field study in natural ecosystems of the Far East revealed a competitive displacement of the native *B. mucronatus* by the invasive *B. xylophilus* (Cheng *et al.*, 2009), which could be attributed to faster population development in the later species (Futai 1980). Considering the observed genetic and phenotypic variation among European populations of *B. mucronatus* we have undertaken a research on interactions between selected strains of *B. xylophilus* (both European and Asiatic) and a series of native Polish isolates of *B. mucronatus* during concurrent invasion and development in the same host.

MATERIALS AND METHODS

In our quarantine glasshouse study, conducted on 2-3-year old seedlings and 20-cm-long logs of *P. silvestris*, reproduction of a single Chinese (Nanjing) and two Portuguese (Mad25c and Pt67OL) strains of *B. xylophilus*, five geographically distant isolates of *B. mucronatus* collected in Poland, and the recently constructed multi-strain intra-specific hybrid of the later species (MT-Rol-01) marked with a Roller *Bmrol-1(mt4)* mutation, were compared when reared separately or in two-species mixed populations. The seedlings were inoculated with a dose of 2500 or 5000 nematodes for single-species cultures, and of 2500 nematodes for each species in mixed populations. In logs the dose was reduced to 500 and 1000 nematodes, respectively. The nematodes were incubated for 1 month at 20°C. Then, the wood was chopped and subjected to water extraction of nematodes. The nematodes were identified based on the shape of female tail terminus. The proportions of phenotypes present in the offspring were counted for each species/strain variant. Selected populations were also subjected to molecular (ITS-RFLP) analysis to confirm the taxonomic status of the offspring.

RESULTS AND DISCUSSION

Phenotypic examination of the nematode offspring revealed that in single-species populations of *B. xylophilus* the range of morphological variation of the female tail has increased when compared to nematodes of the parental populations, which were originally reared *in vitro*, on *Botrytis cinerea*. This was particularly obvious in the Chinese strain (Nanjing), where in 28-42% individuals the tail terminus had a conical projection or a small mucro, compared to mostly broadly rounded terminus of *in vitro*-reared females. The shape of mucro was, however, distinctive from that in *B. mucronatus*. In *B. mucronatus* the female tail was similar in both the nematodes reared *in vitro* and in wood.

In the offspring of two-species mixed populations the proportions of females with *B. xylophilus*- and *B. mucronatus*-like tail generally drifted to one of the parental phenotypes. Interestingly, not only *B. xylophilus* but also *B. mucronatus* could dominate and contribute to significant reduction of the second species. Among five isolates of *B. mucronatus* examined in logs, one (Mdz-1) proved to dominate *B. xylophilus* (Nanjing and Pt67 OL) in 73 and 63% of replicates, respectively, while the isolate Maz-02 dominated these strains in almost 50% of replicates. *B. xylophilus* (Mad 25c) dominated in all experimental variants, however, in individual seedlings or logs these nematodes could also be outperformed by local isolates of *B. mucronatus*.

Insertion of the Roller mutation into the intra-specific hybrid population of *B. mucronatus* (MT-Rol-01) clearly simplified the process of phenotypic examination of the offspring in two-species mixed cultures. By producing the unique phenotype in all developmental stages of *B. mucronatus* the Roller mutation seems to be a very handy marker for any intra- and inter-specific hybridization and controlled rearing of this nematode.

In similar environmental conditions the results of the inter-specific competition in the host differed among species / strain combinations and were apparently related with variation in innate characteristics of the nematode populations. We speculate that the observed phenomenon may have some retarding effect on early success of *B. xylophilus* in colonization of new regions where native populations of *B. mucronatus* are present. These observations need, however, further substantiation in field experiments.

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