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Pathogenicity testing of four *Bursaphelenchus* species on conifer seedlings under greenhouse conditions

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Summary

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer, J. Agric Res. 48, 1934, 949), Nickle (J. Nematol. 2, 1970, 375), is the causative agent of the pine wilt disease and causes serious damage to pine forests around the world. During a survey for the pinewood nematode, four other *Bursaphelenchus* species (*Bursaphelenchus mucronatus*, *B. sexdentati*, *B. anamurius* and *B. vallesianus*) were isolated from wilted pine trees in Turkey. To understand the effects of these *Bursaphelenchus* species on wilting of pine trees, a study was conducted under greenhouse conditions. Two-year-old seedlings of three pine species (*Pinus nigra*, *P. brutia* and *P. pinea*) and one cedar species (*Cedrus libani*) were used. Fifteen seedlings of each species were inoculated with nematodes and 10 seedlings of each species served as controls. The inoculum densities used for each seedling contained approximately 1000 (±100) nematodes of all life stages in 0.25 ml of distilled water. The first wilting symptoms were observed in the fifth week in all pine species but not in the cedar seedlings. All seeding mortality occurred between the 5th and 13th weeks of the study; no mortality was observed outside of this period. The most pathogenic nematode species was *B. mucronatus*, closely followed by the other species. The most susceptible seedling species was *P. nigra,* and *C. libani* was the most resistant species.

1 Introduction

The pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner and Buhrer 1934) Nickle 1970; is considered one of the most serious pests of susceptible conifer forests of the world (Webster and Mota 2008). It is the causative agent of pine wilt disease (PWD) and causes rapid and extensive tree mortality (Mamiya 1983; Kishi 1995). The first record of symptoms of PWD was recorded in Kyushu, Japan by Yano (Yano 1913), and the description of the pinewood nematode as the causal agent of PWD was reported by Kiyohara and Tokushige (1971).

During the last 30 years, PWD has spread to China, South Korea, Taiwan and Portugal (Mota et al. 2009). The increasing geographic range of the pine wilt epidemic has raised concerns that the disease may spread to other Asian and European countries (Webster 1999). The introduction of this nematode into non-native regions has resulted in both extensive wood product losses and dramatic and irreversible changes in forest ecosystems (Mota et al. 2009). Consequently, stricter regulations on the wood product trade and development of new control strategies on the international level are crucial to combat this disease.

Several studies have been conducted on the pathogenicity of *B. xylophilus* and other, congeneric species, under both field and laboratory conditions in several countries. In Italy, Caroppo et al. (2000) inoculated conifer seedlings with three nematode species under open air and controlled conditions and found *B. mucronatus* Mamiya and Enda and *B. sexdentati* Rühm the most pathogenic. Of three nematode species in Greece, Skarmoutsos and Michalopoulos-Skarmoutsos (2000) noted *B. sexdentati* Rühm followed by *B. leoni* Baujard exhibited pathogenicity in a number of pine species seedlings. They found *B. hellenicus* Skarmoutsos, Braasch, Michalopoulos non-pathogenic. In Japan, Mamiya (1999) tested the pathogenicity of *B. mucronatus* on 26-year-old *P. densiflora* Sieb. and Zucc. In Germany, Braasch (1996) found relatively low pathogenicity for *B. mucronatus* on *P. sylvestris* seedlings. On the other hand, Tomminen (1993) observed no mortality of seedlings and field-grown *P. sylvestris* by *B. mucronatus*. Kulinich (2004) conducted several pathogenicity tests and found that *B. mucronatus* was pathogenic to conifer species in some of the tests.

Several studies on the pathogenicity of Norwegian isolates of *B. mucronatus* and *B. xylophilus* on 3-year-old *P. sylvestris* under greenhouse conditions in Norway suggested ambient air temperature affects *B. mucronatus* disease development (Schauer-Blume 1990; Bakke et al. 1991). In Finland, Tarlochan and Sutherland (1989) found the mucronate 'm' isolates of *B. xylophilus* to be less pathogenic that the 'r' form of the nematode in seedlings of *Pinus sylvestris* and *Pinus contorta* Dougl. under greenhouse conditions. Conversely, in Canada, no correlation was found between the 'm' and 'r' forms of the nematode and pathogenicity, although *B. xylophilus* and its hybrids were generally more pathogenic than *B. mucronatus* when *B. xylophilus* or *B. mucronatus* or their intra- or interspecific hybrids from North America, East Asia and Europe were inoculated into greenhouse grown *P. sylvestris* seedlings (Riga et al. 1991). Bolla and Boschert (1993) found a wide range of responses to various isolates of *B. mucronatus* on *P. sylvestris* and *P. strobus* seedlings under greenhouse conditions. In the United States, *B. xylophilus* killed seedlings of the native pine trees *P. banksiana* Lamb. and *P. resinosa* Douglas and exotic Austrian pine, *P. nigra*, but did not kill healthy mature trees (Wingfield et al. 1986). In Japan, Mamiya and Shoji (2009) found Japanese larch, *Larix kaempferi* (Lamb.) Carr. seedlings were susceptible to *B. xylophilus*. Differences in methods and materials in the above studies along with a variety of nematode species and isolates used makes for an indistinct picture of pathogenicity in the pinewood nematode complex. The only clear result is that *B. xylophilus* is the most pathogenic species.

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