Influence of Temperature and Water Supply on Mortality of 3-year-old Pines Inoculated with *Bursaphelenchus xylophilus* and *B. mucronatus*

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**Abstract**

*Bursaphelenchus xylophilus* (US15 and US10 from North America) and *B. mucronatus* (RU-DE-3(w) from Russia and DE-2(w) from Germany) were inoculated each onto 10 three-year-old *Pinus sylvestris* plants and kept at 15, 20, 25 and 30 °C in a climate chamber over three months. Each plant was given 100 ml water twice a week. In a second trial, the effect of various water supplies (20, 50, 80 and 100 % of the maximum water capacity of the soil used) to *P. sylvestris* plants inoculated with the isolates US15 and RU-DE-3(w) was studied. The plants were watered by weighing each plant twice a week until a certain weight was achieved and held at 25 °C in a climate chamber. Each variant contained 10 plants per isolate and 10 control plants. Evaluation based on mortality of the pine plants and numbers of re-isolated nematodes.

In the temperature trial, mortality was 0 at 15 °C in all variants, while it came up to 70 % with US15 at 20 °C, to 100 % with US15 at 25 °C and 30 °C, to 90 % and 70 % with US10 at 25 °C and 30 °C respectively, to 90 % and 30 % with RU-DE-3(w) at 25 °C and 30 °C respectively, and to 20 % and 10 % with DE-2(w) at the same temperatures. Differences in pathogenic action of *B. xylophilus* and *B. mucronatus* were influenced significantly by temperature. The pathogenicity of the German isolate of *B. mucronatus* was very low. *B. xylophilus* was more pathogenic than *B. mucronatus* at all temperatures. In the water trial, the mortality of the plants decreased in both species with increased water supply. The test plants died at a rate of 100 % in both species on the two lowest water levels, while the mortality of control plants was 40 and 10 % respectively. On the third and fourth levels, the mortality was 80 %/70 % and 60 %/60 % for *B. xylophilus* and *B. mucronatus* respectively, while the control plants wilted at a rate of 10 % and 0 % respectively. The number of re-isolated nematodes was higher for *B. xylophilus* than for *B. mucronatus* in all variants.

**Key words:** *Bursaphelenchus xylophilus, B. mucronatus, Pinus sylvestris, environmental factors, temperature, watering, damage*

**Zusammenfassung**

2 Isolate von *Bursaphelenchus xylophilus* (US15 und US10 aus Nordamerika) und 2 Isolate von *B. mucronatus* (RU-DE-3(w) aus Russland und DE-2(w) aus Deutschland) wurden in 4×10 3-jährige *Pinus sylvestris* inokuliert, 3 Monate lang bei 15, 20, 25 und 30 °C in Klimakammern gehalten und mit 2×100 ml Wasser pro Woche bewässert. In einem Versuch zur unterschiedlichen Wirkung der Wasserversorgung auf die Welke der Versuchs-Pflanzen wurden je 10 mit US15 und RU-DE-3(w) inokulierte Pflanzen 2-mal wöchentlich durch Pflanzgefäß-Wägung auf 4 verschiedene Bewässerungsstufen (20, 50, 80 und 100 % der maximalen Wasserkapazität des Bodens) eingestellt und 3 Monate bei 25 °C gehalten. Der Bewertung wurden in beiden Versuchen die Mortalität der Pflanzen im Vergleich zu Kontrollpflanzen und die Anzahl reisolierter Nematoden zugrunde gelegt.

Bei einheitlicher Wasserversorgung starben bei 15 °C praktisch keine Pflanzen ab, bei 20 °C führte nur das *B. xylophilus*-Isolat US15 zum Abwelken von 70 % der Versuchs-Pflanzen, bei 25 und 30 °C starben alle Versuchs-Pflanzen durch *B. xylophilus* US15, 90 bzw. 70 % durch *B. xylophilus* US10, 90 bzw. 30 % durch das russische *B. mucronatus*-Isolat und nur 20 bzw. 10 % durch das deutsche *B. mucronatus*-Isolat. Unterschiede in der Schadwirkung zwischen *B. xylophilus* und *B. mucronatus* wurden signifikant durch die Temperatur beeinflusst. Das deutsche Isolat von *B. mucronatus* zeigte eine ausgesprochen niedrige Pathogenität. *B. xylophilus* war gegenüber *B. mucronatus* bei allen Temperaturen stärker pathogen. Mit steigender Wasserversorgung nahm die Schadwirkung beider Nematodenarten ab. Während bei den beiden niedrigsten Gießstufen nach Inokulation mit *B. xylophilus* und *B. mucronatus* jeweils 100 % der Pflanzen abstarben, betrug die Mortalitätsrate auf der dritthöchsten Gießstufe 80/70 % und bei der höchsten Wasserversorgung 60/60 %. Die Mortalität der Kontroll-Pflanzen betrug in den 4 Gießstufen 40, 10, 10 und 0 %. In allen Varianten wurden nach Inokulation von *B. xylophilus* mehr Nematoden aus den Pflanzen reisoliert als nach Inokulation mit *B. mucronatus*.

**Stichwörter:** *Bursaphelenchus xylophilus, B. mucronatus, Pinus sylvestris*, Umweltfaktoren, Temperatur, Bewässerung, Schaden

**1 Introduction**

Environmental factors influencing pine-wood-nematode-caused mortality of pine trees in natural infection sites and in inoculation tests in the field are chiefly temperature and soil moisture. In Japan, pine trees infected by *Bursaphelenchus xylophilus* in...
spring or autumn wilt slowly and at lower rates than in summer. Mortality also remains low in cool summers. A higher percentage of nematode-inoculated trees wilted at low elevations in Japan than at higher elevations (Kishi, 1995). The yearly changing tree mortality is also thought to be an effect of precipitation. It has also been observed that mortality is highest in warm and dry summers (Rutherford et al., 1990).

In inoculation trials in Japan, three-year-old P. thunbergii inoculated with *B. xylophilus* wilted at constant 25 °C and 30 °C, but did not at 20 °C (Kishi, 1995). In other similar tests, trees wilted at 20 °C and 25 °C but not so at 15 °C. From many experiments, it has been clear that temperatures below 20 °C stopped pine wilting, while higher temperatures promoted it. Other observations have shown that inoculated trees wilted more rapidly under dry soil conditions than in wet locations. The population density of *B. xylophilus* increased less in pine logs with high moisture content than in dryer ones (Kishi, 1995).

It has been shown in many inoculation experiments that not only *B. xylophilus* but also several provenances of *B. mucronatus* have the ability to kill young *Pinus* plants (Baerre et al., 1991; Braasch, 1996; Braasch et al., 1998). Moreover, observations in China, Japan and Russia suggest that trees may have occasionally been killed by *B. mucronatus* in the field (Kishi, 1995; Kulitch et al., 1994). The present inoculation experiments were aimed at showing possible differences in reaction of young trees to inoculation by *B. xylophilus* and *B. mucronatus* at different temperatures and various moisture content of the soil.

### 2 Material and methods

The inoculated plants were three-year-old *Pinus sylvestris* potted at least six weeks before inoculation in pots of 10 cm in diameter. The substrate used was peat in which 5% sand, 6% cocopeat and 15% clay were mixed. The inoculation was executed by cutting a 1-cm-long slit on the stem of the young plants, just below the new shoot, inserting a small cotton strip followed by dropping 1/2 ml of water containing 6000 nematodes, and sealing by an adhesive strip as described by Braasch (1996). Inoculations were executed after plants had had their shoots in early summer. The plants had been held in a greenhouse some weeks before.

The plants for the temperature test were split up in four sets of 50 plants and held under constant 15 °C, 20 °C, 25 °C and 30 °C. Fourty plants of each set were inoculated with four different nematode strains, i.e. 10 plants per strain, and 10 control plants were inoculated with water only. Each plant was given 100 ml of water twice a week. In the water trial, plants were split up in four groups of 30 plants. In each group, 20 plants were inoculated with two different nematode strains, i.e. 10 per strain, while the remaining 10 plants were inoculated with water and served as a check. The four groups of plants were watered differently by weighing each plant twice a week until a certain weight was achieved, according to four levels of the soil’s water capacity. The average amounts of water used per week and plant were 113, 188, 290 and 365 ml on the four levels, respectively. This came up to approximately 20, 50, 80 and 100% of the maximum water capacity of the soil used. The inoculated and control plants were held at 25 °C.

The plants were observed for symptoms every week. Dead plants were cut immediately above the ground, twigs and needles were removed and the stems were chopped and extracted by Baermann funnel technique for 48 hours to gain the nematodes. Plants that had not died off totally were cut, chopped and extracted after three months.

Nematodes used for inoculations in the first trial were *B. xylophilus* US15 (round-tailed form) from Illinois, United States, *B. xylophilus* US10 (mucronate form) from Minnesota, United States, *B. mucronatus* DE-2(w) from Grünberg, Germany, and *B. mucronatus* RU-DE-3(w) from Novosibirsk, Russia. The two *B. mucronatus* isolates were selected because of their different pathogenicity that had been shown in previous inoculation experiments. In the second trial aimed at investigating the influence of watering, only the two more pathogenic isolates of the two species, US15 and RU-DE-2(w), were used.

### 3 Results

As shown in Tab. 1, very few nematodes of both *B. xylophilus* and *B. mucronatus* were found in inoculated pines kept at 15 °C over three months, and tree mortality was in no case higher than in the control plants. Concluding from the number of re-isolated nematodes (Tab. 1), *B. xylophilus* US 15 obviously caused 70% mortality of the young plants at 20 °C, whereas US 10 and the *B. mucronatus* isolates failed to develop well and cause mortality at this temperature. The temperature most suitable for the strains investigated seems to be 25 °C, as three of the isolates caused high mortality (Tab. 1), while only the German isolate of *B. mucronatus* caused no mortality and had a very low multiplication rate at all of the four temperatures. The Russian isolate of *B. mucronatus* acted in a similar way as the two *B. xylophilus* isolates, although its multiplication rate was somewhat lower. At 30 °C, only the US 15 isolate was very successful (100% mortality), whereas the mortality observed in plants with the US 10 isolate could obviously not only be traced back to the presence of the nematodes.

Fig. 1a-d show the number of wilted trees depending on the weeks after inoculation at the four temperature levels. The earlier the plants start to wilt, the higher the mortality finally observed will be.

Results of inoculation experiment executed with a pathogenic strain of both *B. xylophilus* and *B. mucronatus* under various water supply (Tab. 2) and at constant 25 °C show that the pines wilted completely on levels 1 (lowest watering) and 2, at 80% on level 3 and at 60% on level 4. Control plants wilted at 40% on level 1 due to water stress. The number of re-isolated nematodes per gram of wood was again higher in the case of *B. xylophilus* than in *B. mucronatus* on all water levels. In both cases, the number of re-isolated nematodes increased from water level 1 to 3, but decreased on water level 4. Trees wilted more rapidly in dry soil than in wet soil (Fig. 2a and b).

Statistical analysis was done by analysis of variance, Tukey test procedure (significance level 0.05) for the temperature experiment. Significant differences in numbers of re-isolated nematodes were shown between US15/US10 and US10/DE-2(w) at 15 °C, US15/US10, US15/DE-2(w) and US15/RU-DE-3(w) at 20 °C, US15/ RU-DE-3(w) at 20 °C and 25 °C. No significant differences were obvious, but there was a clear numeric difference for the US15 isolate held at 20 °C and at other temperatures, which could not be shown to be significant. T-test (α = 0.05) was used to show significant differences between the number of nematodes extracted in the water experiment. Differences between the two isolates were significant on water level 2, while numeric differences within the isolates on various water levels were significant only for *B. xylophilus* between water level 1 and water level 3.

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Fig. 1a. Inoculation of 3-year-old *P. sylvestris* plants with *B. xylophilus* and *B. mucronatus* strains at 15°C (n = 10)

Fig. 1b. Inoculation of 3-year-old *P. sylvestris* plants with *B. xylophilus* and *B. mucronatus* strains at 20°C (n = 10)

Fig. 1c. Inoculation of 3-year-old *P. sylvestris* plants with *B. xylophilus* and *B. mucronatus* strains at 25°C (n = 10)
Fig. 1d. Inoculation of 3-year-old *P. sylvestris* plants with *B. xylophilus* and *B. mucronatus* strains at 30 °C (n = 10)

Fig. 2a. Inoculation of 3-year-old *P. sylvestris* plants with *B. xylophilus* US 15 at 25 °C and various water supply

Fig. 2b. Inoculation of 3-year-old *P. sylvestris* plants with *B. mucronatus* RU-DE-3(w) at 25 °C and various water supply

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4 Discussion

The results show that the US15 isolate of *B. xylophilus* caused the highest mortality of three-year-old *P. sylvestris* plants compared to the other isolates investigated in both experiments. They support previous observations that *B. xylophilus* may act as a pathogen at mean July/August temperatures from 20 °C upwards, while *B. mucronatus* may only act as a pathogen at higher temperatures. BRAASCH (1997) also observed a higher pathogenicity of *B. xylophilus* compared to *B. mucronatus* in trials in cages under outdoor conditions in Germany in a warm summer (July/August average temperature 21.1/19.5 °C at experimental place).

Previous observations on the different pathogenicity of various *B. mucronatus* isolates and the apathogenic feature of the German DE-2(w) isolate were confirmed. Pathogenicity of *B. mucronatus* was only observed in the case of the Russian isolate at 25 °C. Most probably, the isolate RU-DE-3(w) needs a higher day degree sum than *B. xylophilus* to achieve a population level that kills a young tree. It is hard to find an explanation for the decrease of mortality and nematode numbers at 30 °C in the case of RU-DE-3(w). The best temperature to evaluate the pathogenic properties of an isolate seems to be 25 °C.

Soil water deficit enhanced rapid progress of disease symptoms and was related to high mortality in inoculated pine plants. Although virulent isolates can kill even trees without water-stress, some trees did not wilt under moderate soil moisture. Whereas temperature range influenced the action of *B. xylophilus* and *B. mucronatus* differently, the two isolates inoculated caused about the same mortality at different water levels at 25 °C. However, *B. xylophilus* had again a higher multiplication rate than *B. mucronatus*, which was shown to be significant on water level 2. The death of pines caused by pine wilt disease is related to the death of parenchyma cells as well as to the decrease in hydraulic conductance of the xylem (IKEDA and KYOHARA, 1995). Obviously, dry-suffering trees are killed by fewer nematodes than others, and water status of plants influences proliferation of the nematodes.

Inoculation experiments show very well if a nematode species or provenance has a pathogenic potential or not and which factors influence development and action of the nematodes. However, transfer of results to grown-up trees must be applied carefully. A special combination of various factors is obviously required for the development of pine wilt disease. Although the isolates of *B. xylophilus* and *B. mucronatus* investigated provoked a very similar reaction on the young trees under respective watering and constant 25 °C, they may act differently in nature due to specific temperature sums necessary for their development and to different circumstances necessary to overcome the tree’s defense system. The relationship among pathogenicity of an isolate, the physiological condition of the pines and the environmental conditions must be considered. It is obvious that the epidemic pathogenic action of *B. xylophilus* on pines outside its region of origin (North America) is much more drastic than the occasionally observed damaging effect of *B. mucronatus*. However, the existence of provenances of *B. mucronatus* showing differences in pathogenic potential must be considered. *B. mucronatus* is a very heterogeneous species (BRAASCH et al., 1998), and not much is known on the action of its geographic strains on conifers outside their distribution area. Additionally, the environmental conditions as a whole, that are correlated with a pathogenic action of *B. mucronatus* are not fully known.

IKEDA and SUZAKI (1984) have shown that there are differences in pine reactions to infection by virulent and avirulent *B. xylophilus* isolates. While the water conducting function of xylem in pines inoculated with a virulent strain was lost completely, the hydraulic conductance of stem and root systems decreased in pines inoculated with an avirulent strain, but was not completely.

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lost. Some strains of *B. mucronatus* cause similar symptoms in young trees as observed with pathogenic *B. xylophilus*, and their effect may be classified as between virulent and avirulent *B. xylophilus* strains. The results of the described experiments lead to the conclusion that differences between the effects of the *B. xylophilus* and *B. mucronatus* strains investigated are decisively determined by temperature.

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Literature


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Sortenspezifische Unkrautunterdrückung bei Kartoffeln und deren Veränderung durch eine N-Unterfußdüngung

Effects of cultivar and N-placement on the weed suppressing potential of potato stands

Von Peter Niemann

Zusammenfassung


Von den acht Sorten des engeren Testsortiments waren Atica (sehr früh), Cilenia (früh), Paola (mittelfrüh) und Donella (spät) gegenüber einer simulierten Spätenkunraftung mit *Solanum nigrum* konkurrenzschwach, wohingegen Rita (sehr früh), Ute (früh), Fausta (mittelfrüh) und Indira (spät) als konkurrenzstark einzustufen sind. Im Durchschnitt der Sorten und Jahre wurde das Unkrautwachstum durch die konkurrenzstärkeren Sorten um 39% reduziert. Eine N-Reihendüngung bewirkte gegenüber der Flächendüngung einen Unkrautunterdrückungseffekt von 50% bei den geprüften Sorten Paola und Fausta. Der kumulative Effekt von Sortenwahl und Reihendüngung erreichte bei Gegenüberstellung dieser beiden Sorten 81%. Im Gesamtsortiment war das Merkmal Unkrautunterdrückung wie folgt verteilt: Die Hälfte der über 200 Sorten zeigte eine mittlere Merkmalsausprägung, ein Viertel wich negativ vom Mittelfeld ab und ein weiteres Viertel positiv. Damit liegen züchterische Ansatzpunkte für eine weitere Verbesserung der sortengebundenen Unkrautunterdrückung bei Kartoffeln vor.

Stichwörter: Kartoffeln, Sorten, Unkraut, Reihendüngung