Journal of Nematology 23(2):188–193. 1991.
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Influence of Temperature on Development of Pine Wilt in Scots Pine[1]

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Abstract. The effect of temperature on pine wilt development in Scots pine (*Pinus sylvestris*) was examined in three experiments. Container-grown pines (4–6 years old) inoculated with 1,500 *Bursaphelenchus xylophilus* were incubated at constant temperatures in growth chamber for 8 weeks, then at a temperature range of 15–30 C in a greenhouse for 10–12 weeks. Nematode infection was greater, tree mortality was higher, and disease incubation was shorter at 32 and 30 C than at 25, 23, 18, and 11 C. Foliar symptoms developed more rapidly and uniformly at higher temperatures. Ninety-five percent of tree deaths at 32 and 30 C and 88% at 25 and 23 C occurred within the 8-week exposure to constant temperatures. Mortality at 18, 16, and 11 C occurred only after transfer to the greenhouse. Results indicate that pine wilt incidence is directly related and disease incubation period is inversely related to temperature and that high-temperature stress predisposes Scots pine to lethal infection by *B. xylophilus.*
Key words: Bursaphelenchus xylophilus, pine, pine wilt, pinewood nematode, *Pinus sylvestris,* Scots pine, temperature.

Pine wilt disease, caused by the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle, was first recorded in Illinois in 1979 and rapidly became epidemic there in the early 1980s (4). Losses in some large plantings of Scots pine, *Pinus sylvestris* L., and Austrian pine, *P. nigra* Arnold, have exceeded 25%. Although *B. xylophilus* apparently is indigenous to most of North America, the distribution of pine wilt is much less widespread (1,4). Symptoms typical of the disease have been noted only in portions of the Midwest, East Coast, Southeast, and Southwest. The most intense incidence of pine wilt occurs in the central Midwest, where highly susceptible Scots and Austrian pines have been planted extensively as landscape, windbreak, and Christmas trees.

Environmental factors may directly influence distribution and intensity of pine wilt in North America (9), as they apparently do in Japan in native pine species (6–8,10). The annual losses in forests in many areas of Japan correlate with high temperatures and low precipitation that occur during the growing season (8). The disease is rare or nonexistent in areas of minimal abiotic stress on pines. In Illinois, where year-to-year summer climatic conditions fluctuate widely, summer–autumn incidence of the disease has been more extensive and symptom development more rapid in years with high summer temperatures and moisture stress on pines (4). Thus, stressful summer weather conditions during the growing season appear to favor spread and development of the disease (8). The objective of our study was to determine the influence of temperature on development of pine wilt in the widely planted and highly susceptible Scots pine under controlled conditions.

Materials and Methods

The relationship of ambient temperature to infection by *B. xylophilus,* incidence of pine wilt, and incubation period of the disease in Scots pine was studied in three annual experiments. *Bursaphelenchus xylophilus* was isolated from an infected Scots pine on the University of Illinois campus in Urbana. Nematodes were surface disinfested by soaking in a 0.5% aqueous solution of sodium ethylmercurithiosalicylate for 3 hours and rinsing in distilled water (11). Populations were increased monoxenically on the fungus *Botrytis cinerea* Pers. on potato dextrose agar in 40-ml culture tubes at 21–24 C. Test trees were Scots pines, 4–6 years old, 35–80 cm high, and

Received for publication 7 July 1989.

[1] This research was part of Project 68-382 of the Agricultural Experiment Station, College of Agriculture, University of Illinois at Urbana–Champaign.

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growing in 8-liter plastic containers. They were divided into equivalent groups with similar ranges of tree size for temperature treatments and then into subgroups for nematode inoculation and control treatments. Trees were inoculated when candles were fully elongated and new-growth needles were about one-half elongated. One or two lateral branches were excised 4 mm from the main stem at mid height. A 9-cm-long segment of rubber tubing, 4 mm ID, was affixed to the twig butt remaining and supported at a 60-degree angle by tape attached to the stem. A 1-ml aerated water suspension of 750 or 1,500 axenic nematodes was injected into the tube with a sterile syringe. The distal end of the tube was sealed with tape to prevent evaporation and air-borne contamination. Control trees were inoculated similarly with a nematode-free water suspension of propagules from stock cultures of *B. cinerea.* Trees were arranged randomly in constant temperature growth chambers with a 14-hour day length, incubated for 8 weeks, and watered when the soil surface was dry.

The onset of foliar symptom development, detectable candle wilt and (or) color change of all or part of the tree, was recorded for individual trees. Dead trees, with an overall yellowish-brown cast, were removed and analyzed for *B. xylophilus* by sectioning main stems and incubating the wood in tap water overnight. Extracted nematodes were quantified by determining numbers in five 1-ml aliquots from 100-ml suspensions. Sample wood then was oven dried at 80 C for 3 days, and nematode population density was calculated on the basis of number of nematodes per gram of dried wood. Eight weeks after inoculation, asymptomatic trees were transferred to a greenhouse to continue incubation at 15–30 C. Subsequent tree mortality was recorded and wood was analyzed as described here. When experiments were terminated, remaining nematode-inoculated trees and three of five control trees in each temperature treatment were analyzed for the nematode.

Experiment 1: Four-year-old Scots pines, cv. East Anglica, were inoculated with 1,500 nematodes or fungus alone at a single site per tree. Trees were retained in a laboratory at 21 C for 48 hours to allow nematode infection of the inoculation site, then were incubated in growth chambers at 16, 23, or 30 C for 8 weeks. Seven nematode-inoculated and five control trees comprised each temperature treatment. After the 8-week exposure to constant temperatures, live trees were incubated in the greenhouse for 10 weeks.

Experiment 2: Four-year-old Scots pines, cv. Belgian, were inoculated with 1,500 nematodes or fungus alone at a single site per tree, then placed in growth chambers at 11, 18, 25, or 32 C immediately after inoculation. Ten nematode-inoculated and five control trees comprised each temperature treatment. Because of low disease incidence in chambers during the initial 8 weeks, subsequent greenhouse incubation was extended to 12 weeks.

Experiment 3: Six-year-old Scots pines, cv. East Anglica, were inoculated with 750 nematodes or fungus alone at each of two adjacent sites on each tree. Transmission by vector beetles is presumed to occur at more than one site on a tree under natural conditions, and the dual inoculation per tree increased infection potential. Trees were placed in growth chambers at 16, 23, or 30 C immediately after inoculation. Ten nematode-inoculated and five control trees comprised each temperature treatment. After the 8-week exposure to constant temperatures, surviving trees were incubated in the greenhouse for 12 weeks.

Results

Incidence of pine wilt was highest and symptom development was most rapid and uniform in Scots pines exposed to 30 or 32 C constant temperatures (Table 1). Disease incubation generally was longer and symptom development was slower at lower temperatures. Except in experiment 3, tree mortality was less than 100% in all temperature treatments, consistent with results of our earlier research under greenhouse conditions. Most mortality at 23 C

TABLE 1. Mortality of 4–6-year-old Scots pines over a 20-week period at different temperatures after inoculation with 1,500 *Bursaphelenchus xylophilus.*

Initial temperature treatment (C)	Dead or dying trees, 3–20 weeks after inoculation												Dead trees
	Constant temperature chamber						Variable temperature greenhouse†						
	3	4	5	6	7	8	10	12	14	16	18	20	
	Experiment 1‡												
16	0	0	0	0	0	0	1	0	1	0	1	—	3
23	0	1	1	1	0	0	0	0	0	0	0	—	3
30	0	2	1	1	1	1	0	0	0	0	0	—	6
	Experiment 2§												
11	0	0	0	0	0	0	0	0	0	1	0	0	1
18	0	0	0	0	0	0	0	0	0	1	0	0	1
25	0	0	1	0	0	0	0	0	0	0	0	0	1
32	2	1	1	0	0	0	0	0	0	0	0	0	4
	Experiment 3‖												
16	0	0	0	0	0	0	0	0	1	3	0	0	4
23	0	0	1	0	2	0	0	0	0	1	0	0	4
30	7	2	0	0	0	0	0	1	0	0	0	0	10

† Greenhouse temperatures ranged from 15 to 30 C.
‡ Each constant temperature treatment contained seven nematode-inoculated and five control trees; one control tree died in each temperature treatment but contained no nematodes.
§ Each constant temperature treatment contained 10 nematode-inoculated and 5 control trees; one control tree died at 32 C but contained no nematodes.
‖ Each constant temperature treatment contained 10 nematode-inoculated and 5 control trees; no control trees died.

and above occurred during the initial 8-week exposure to constant temperatures. At 18 C and below, symptom development and tree death occurred only after trees were transferred to the warmer greenhouse. Stems of dead, nematode-inoculated trees contained *B. xylophilus* in population densities of 869–26,119 nematodes/g dried wood. Though density could not be correlated with temperature treatment, highest populations usually were extracted from high-temperature treated trees. No nematodes were found in any control trees analyzed.

Experiment 1: Six of seven nematode-inoculated trees at 30 C and three at 23 C died during incubation at constant temperatures (Table 1). The first mortalities occurred 4 weeks after inoculation in both treatments. Three trees in the 16-C treatment died after transfer to the greenhouse. No further incidence of disease occurred in other treatments. Nematode population densities averaged 4,099, 6,711, and 2,590/g dried stem wood at 30, 23, and 16 C, respectively. One asymptomatic tree in the 16-C treatment contained a few nematodes in the stem, but remaining nematode-inoculated trees were uninfected 18 weeks after inoculation. One control tree, weakened earlier by winter injury, died in each temperature treatment but did not contain nematodes.

Experiment 2: Four of ten nematode-inoculated trees at 32 C died, the first two at 3 weeks after inoculation (Table 1). Only one of the trees at 25 C died during incubation at constant temperatures. Two trees, one in 18-C and one in 11-C treatment, died after transfer to the greenhouse. Nematode population densities averaged 20,244, 3,627, 5,122, and 10,182/g dried stem wood at 32, 25, 18, and 11 C, respectively. A single branch flag occurred above the point of inoculation on one tree in the 25-C treatment and multiple flags occurred on one tree in the 18-C treatment during greenhouse incubation, but symptoms progressed no further. The flags were similar to those observed in infected established trees under natural conditions during late autumn and early spring. *Bursaphelenchus xylophilus* was not present in stems of flagged trees, but nematodes were extracted in low numbers from the flag branches at 20 weeks after inoculation. Remaining nematode-inoculated trees were uninfected at 20 weeks. One initially weak control tree at 32 C died, but it contained no nematodes.

Experiment 3: All 10 nematode-inoculated trees in the 30-C treatment died, nine during incubation at the constant temperature and within 4 weeks after inoculation (Table 1). Four trees at 23 C died, three at 5–7 weeks after inoculation. Four trees in the 16-C treatment died, but not until transfer to the greenhouse. Nematode population densities averaged 16,203, 5,610, and 6,142/g dried stem wood at 30, 23, and 16 C, respectively. One asymptomatic tree in the 23 C treatment contained fewer than 100 nematodes in the stem extract. Remaining nematode-inoculated trees were uninfected 20 weeks after inoculation. No control trees died during the experiment.

Discussion

Results show that incidence of pine wilt and speed and uniformity of symptom development in Scots pine are directly related to ambient temperature. Only *B. xylophilus*-inoculated trees initially incubated at 23–32 C died during the 8 weeks at constant temperatures. Both nematode infection and incidence of disease were highest at 30–32 C. Infections occurred in some trees at temperatures below 23 C, but death occurred only after trees were transferred to the greenhouse, where diurnal temperatures frequently exceeded 30 C. Dead or dying infected trees browned more rapidly at temperatures above 23 C. Development of foliar symptoms at the higher temperatures was relatively uniform throughout the tree, whereas development at the lower temperatures was asynchronous, trees expressing various stages of browning in the crown. The disease incubation period was inversely related to temperature. Most deaths occurred within 3–5 weeks at 30–32 C, 4–6 weeks at 23–25 C, and 14–16 weeks at 11–18 C. Mortality of the inoculated Belgian pines was relatively low, even at high temperatures. The low disease incidence may have been a reflection of resistance conferred by the sturdier condition of those trees or of a degree of intrinsic resistance in this cultivar.

Development of symptoms and variations in symptom expression in the young Scots pines at the temperatures tested were identical to those observed in pine wilt incidence in older trees under natural conditions in Illinois (4). Symptoms develop rapidly and uniformly during the late summer–early autumn period of mortality, following the high-temperature stress of midsummer and rapid increase and dispersion of nematode populations within infected trees. In contrast, symptoms in late autumn and spring mortalities generally develop slowly and asynchronously, in association with lower seasonal temperatures. Flagging and nematode population restriction to flags, encountered in the field in late fall and early spring, were reproduced in two test trees incubated at lower temperatures and in several trees inoculated in late summer in related research (5).

The influence of temperature on pine wilt development in Scots pine under controlled conditions was similar to that reported from Japan in young native pines (2,3). Exposure of *B. xylophilus*-infected Japanese red pines (*P. densiflora* Sieb. & Zucc.) to 15 or 20 C inhibited disease development, whereas incubation at 25 or 30 C induced typical symptoms and tree death (3). Exposure of trees to 18 C after initial incubation at 30 C also inhibited disease development, but a return to warmer temperatures stimulated development. Inoculated Japanese red pines became infected at 18 C, but no disease developed until trees were transferred to 30 C (2).

Results of this and Japanese research confirm field observations that high-temperature stress is a major abiotic factor physiologically predisposing susceptible species of pines to lethal infection by *B. xylophilus*; i.e., expansion of the nematode population through the tree from initially localized sites of infection (transmission sites) culminating in tree death. Data suggest a temperature threshold of approximately 21–23 C for lethal infection. Daily mean temperatures normally exceed that threshold during the summer months throughout Illinois and in portions of neighboring prairie states (12), where susceptible pines have been planted extensively and where pine wilt became epidemic. During normal summers in Illinois, pines often are subjected to extended periods of high thermal stress, in which maximum daily temperatures reach or exceed 30 C. Incidence of pine wilt in our study was greatest under experimentally induced, similar thermal stress conditions. Normal to above-normal temperatures characterized the Illinois summers of 1980, 1982, and 1984, years in which incidence of pine wilt was high during the late summer to fall mortality period (4, unpubl. observations). In contrast, the summers of 1981, 1985, and 1986 had well-below-average temperatures and few high-stress days. Late-season incidence of the disease subsequently was very low in those years, but mortality in the springs of 1982, 1986, and 1987 was relatively high, apparently as a result of temperature-related, delayed completion of the disease cycle. Moisture stress frequently accompanying summer thermal stress in Illinois also may increase susceptibility to the disease in Scots and other pines in the region, as demonstrated with Japanese black pine (*P. thunbergii* Parl.) in Japan (10). Though incidence in drought years without serious temperature stress has been low in Illinois, moisture stress may enhance the predisposing influence of temperature and lower the thermal threshold for lethal infection.

Incidence of pine wilt appears to terminate just north of Illinois, with a sharp gradient zone between regions of high and no mortality. Classic symptoms of the disease cannot be found in either native or introduced pines in Minnesota, Wisconsin, or Canada (13) or in northern New England (Malek, unpubl.), even though *B. xylophilus* and its known and potential vectors in the genus *Monochamus* are endemic to most of those regions. The line of demarcation roughly coincides with the extrapolated mean 22-C temperature isotherm for July (9,12), the warmest month of the summer in North America, as well as with the southern border of the natural northern pine belt. Above that line, *B. xylophilus* is associated only with dead pines killed by other factors and with dead branches on live trees stressed by insects and fungi (13,14). Under conditions of minimal annual summer abiotic stresses, even live susceptible pines, such as Scots and Austrian pines, apparently can resist invasion by the nematode. A recent climatic analysis by Canadian scientists has revealed further positive relationships between summer temperatures and incidence of pine wilt, where *B. xylophilus* does or could occur in the northern hemisphere (9). The observed distribution of pine wilt and results of experimental research, therefore, provide strong evidence that the northern limit of incidence is climatically governed and that the disease indeed poses minimal threat to the vast area of natural forest and plantation pines of the northern pine belt.

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