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Change in Water Status of *Pinus thunbergii* Parl. Inoculated with Species of *Bursaphelenchus*

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Abstract: Maximum and minimum xylem pressure potentials of needles were measured to evaluate water status of *Pinus thunbergii* Parl. after inoculation with the virulent or avirulent populations of *Bursaphelenchus xylophilus* or *B. mucronatus*. In virulent *B. xylophilus*-inoculated pines, the water status changed abruptly and needle chlorosis occurred by day 29 after inoculation. Similar changes were not seen in *B. mucronatus*-inoculated and uninoculated control pines. Oleoresin flow ceased in virulent *B. xylophilus*-inoculated pines. Avirulent *B. xylophilus*-inoculated pines responded very little to nematode invasion by a slight decrease in oleoresin flow. Oleoresin flow did not vary in *B. mucronatus*-inoculated and uninoculated control pines. A decrease in soil water potential below field capacity seemed to accelerate the development of pine wilt disease.

Key words: avirulent population, *Bursaphelenchus mucronatus, B. xylophilus,* oleoresin flow, pine wilt disease, pinewood nematode, *Pinus thunbergii,* soil water potential, virulent population, water status, xylem pressure potential.

The relationships between pine wilt disease caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle, and water status of pine is fairly clear (2,5,10,11). Genotypically different host specific pathotypes (1) and populations (6,7) of *B. xylophilus,* differing in virulence, have been identified and compared biochemically (1). There are, however, no comparative studies on water relations in pine trees inoculated with avirulent, virulent, or nonpathogenic populations.

The objective of our research was to determine the influence of two populations of PWN, differing in pathogenicity, on the water status in pines. The effects of environmental conditions such as soil water potential and climate on the development of pine wilt disease also were considered.

MATERIALS AND METHODS

Plant material and inoculation with nematodes: Three-year-old Japanese black pine, *Pinus thunbergii* Parl., average height 113.5 cm and basal diameter 2.9 cm, growing under field conditions at Kyushu Research Center, Kumamoto, Japan, were used. Stand density was three pines per square meter. On 13 July 1988 second-year growth on a branch between the second and third internodes was inoculated with 10,000 virulent (S6-1 population) (Bxv) (7) or avirulent (C14-5 population) (Bxa) *B. xylophilus,* or *Bursaphelenchus mucronatus* Mamiya and Enda (Bm) (8). Controls were treated with sterile water. Fifteen seedlings were used for each treatment.

Symptom development and oleoresin flow: Disease development in the inoculated seedlings was examined periodically by evaluating oleoresin flow and visual symptoms. The amount of oleoresin which accumulated after several hours from a hole drilled through the bark into the sapwood of all tested seedlings was classified in four categories: 1 = no flow, 2 = appreciable flow, 3 = copious but not overflowing, 4 = copious and overflowing. Inoculated and control pines were harvested 2 months after inoculation. Samples from the current shoot, treated branch, and basal part of stem were cut into small pieces, and nematodes were extracted in a Baermann funnel. The number of nematodes extracted in 24 hours was recorded. Dry weights of wood pieces were determined after drying at 80 C for 48 hours.

Water status of pines: To determine the changes in water status of pine seedlings after inoculation with nematodes, the base xylem pressure potential (BP) and the maximum xylem pressure potential of needles

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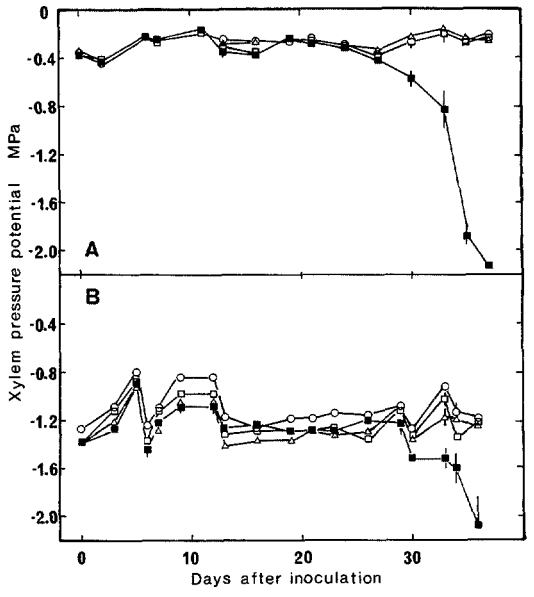

FIG. 1. Changes in base and minimum xylem pressure potentials in inoculated pine seedlings. A) Base xylem pressure potential (BP) measured just before sunrise. B) Minimum xylem pressure potential measured during midday. ■ = virulent *B. xylophilus*-inoculated pines. □ = avirulent *B. xylophilus*-inoculated pines. △ = *B. mucronatus*-inoculated pines. ○ = controls. Values are reported as the mean ± SE, N = 5. Vertical lines indicate SE. Line missing in the figure is due to larger symbols than SE.

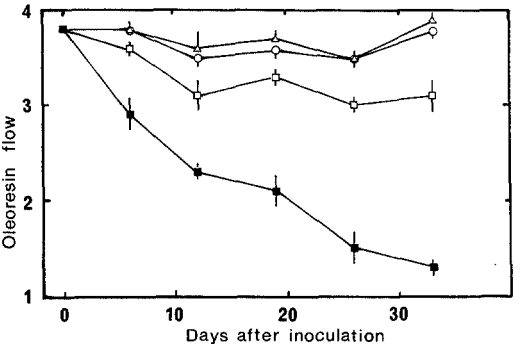

FIG. 2. Changes in oleoresin flow in inoculated pines. ■ = virulent *B. xylophilus*-inoculated pines. □ = avirulent *B. xylophilus*-inoculated pines. △ = *B. mucronatus*-inoculated pines. ○ = controls. Values are reported as the mean ± SE, N = 15. Vertical lines indicate SE.

were measured using a pressure chamber. Measurements of BP and the minimum xylem pressure potential of five tested pines for each treatment were made just before sunrise (4:00 to 5:00 a.m.) and during midday (1:00 to 2:00 p.m.).

Environmental conditions: Soil water potential was measured at depths of 10, 20, and 30 cm at five locations per depth with mercury manometer soil tensiometers at 1:00 p.m. Root systems of pine trees mainly were located at 7–20 cm deep.

Climatic data such as air temperature, vapor pressure deficit (VPD), and precipitation were obtained from the monthly report of Kumamoto Meteorological Station, situated 2 kilometers west of the study site.

RESULTS

Nematode detection: All of the Bxv-inoculated pines died and PWN was recovered from the current shoot, inoculated branch, and basal part of stem. Nematode population levels ranged from 300 to 4,500 nematodes per gram dry weight of wood. Bxa-inoculated or Bm-inoculated pines did not die and no PWN were recovered.

Water status: The BP values ranged from −0.16 to −0.43 MPa until day 29 after inoculation (Fig. 1), and there was no significant difference between inoculated and control pines. Subsequently, however, the BP decreased drastically in Bxv-inoculated pines but did not change in Bxa-inoculated and Bm-inoculated pines. By day 30 the minimum xylem pressure potential decreased in Bxv-inoculated pines but was unaffected in other treated pines.

Oleoresin flow and needle color: Oleoresin flow (Fig. 2) did not vary in Bm-inoculated and control pines, whereas in Bxv-inoculated pines it decreased steadily after inoculation and finally ceased. In Bxa-inoculated pines the oleoresin flow was reduced slightly by day 12 after inoculation.

Needle color of Bxa-inoculated and Bm-inoculated pines was unchanged, whereas in Bxv-inoculated pines chlorosis of previous-year needles was observed by day 30 after inoculation.

Climatic conditions and soil water status: During the first 2 weeks after the pines were inoculated, the accumulated precipitation was twice the 1951–1980 average amount (Fig. 3). The soil water potential

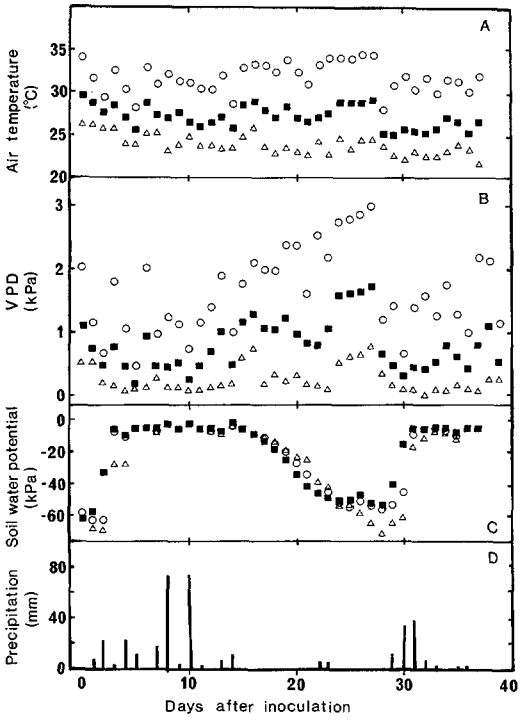

FIG. 3. Diurnal changes in climatic conditions and soil water potential. A) Air temperature. ○ = maximum temperature. ■ = mean temperature. △ = minimum temperature. B) Vapor pressure deficit (VPD). ○ = maximum VPD. ■ = mean VPD. △ = minimum VPD. C) Soil water potentials measured at depths of 10 cm (○), 20 cm (■), and 30 cm (△). Values are reported as the mean ± SE, N = 5. Vertical lines indicating SE are missing because SE is smaller than symbols. D) Precipitation.

was over −0.01 MPa, and it never fell below field capacity of −0.06 MPa (Fig. 3). The VPD was low. Air humidity in the experimental field also was high during the first 2 weeks. After 2 weeks rainfall was limited; the soil water potential decreased gradually until day 23, after which it remained constant at about −0.06 MPa until day 29; and the VPD increased.

DISCUSSION

Pinus thunbergii showed different reactions to infection by *Bursaphelenchus* spp. Avirulent *B. xylophilus* (C14-5) did not establish in *P. thunbergii,* even though it induced a slight reduction in oleoresin flow.

The abrupt decrease in water status of Bxv-inoculated pines corresponded to the appearance of chlorosis in previous-year needles. This agreed with other reports (4,5,11), although the onset occurred 8 days later than in some studies (4,5). This discrepancy may be due to a difference in climatic conditions and (or) soil water status. Below −0.06 MPa of soil water potential, resistance to water movement from soil to root system increases; below −0.1 MPa, water absorption by plants is restricted (3). Frequent rain during the first 2 weeks after inoculation kept the soil water status high and the VPD, which is the most important factor for controlling transpiration, was low. Thus experimental pines experienced wet soil conditions, water absorption was not restricted, water loss by transpiration was suppressed, and there was no water stress. During the study by Ikeda and Suzaki (5), rain was infrequent and soil water potential ranged from −0.05 to −0.07 MPa. This indicates that absorption by pines was slightly restricted and water stress was moderate. Mortality rate of PWN-inoculated pines grown under −0.03 MPa soil water potential was very low (10). The increase of nematode population level in wood is influenced by water status of pines (9–11). Soil water potential of −0.06 MPa does not disturb physiological conditions, such as photosynthesis and water relations in healthy trees, nor does it lead to reduced growth or death. For pine wilt disease to develop in pines infected with virulent PWN, however, the soil water potential apparently must fall below field capacity.

In avirulent population-inoculated pines slight reduction of oleoresin flow occurred and PWN did not live. In contrast, in Bm-inoculated pines as well as uninoculated control pines reactions such as water status, oleoresin flow, and needle chlorosis were not found. The population level of another avirulent *B. xylophilus* (Ok-2), which is more virulent than C14-5 used in this study, decreased in pine trees after inoculation (7). Pine trees apparently react, particularly in oleoresin physiology, to the invasion of an avirulent *B. xylophilus* population, but the life or death of pines inoculated with avirulent *B. xylophilus* and the reproduction of

the PWN apparently depend on the host vigor in relation to environmental conditions such as soil water status and climatic conditions.

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