

Enhancement of *Sphaeropsis sapinea* Stem Invasion of Pines by Water Deficits

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ABSTRACT

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Three-year-old Austrian, Scots, and Japanese black pines growing in a loamy clay soil were inoculated with *Sphaeropsis sapinea*. Water was withheld for periods sufficient to create soil water potentials of -0.1, -0.6 to -0.8, and -1.2 to -1.5 MPa. Increasingly negative soil water potentials promoted greater linear fungal growth in stems. Scots and Austrian pines were more susceptible to *S. sapinea* than Japanese black pine, although fungal growth within stems of this normally resistant species was extensive. These findings support field observations of increased infection by *S. sapinea* on droughted or unhealthy trees.

Tip blight caused by *Sphaeropsis sapinea* (Fr.) Dyko & Sutton (*Diplodia pinea* (Desm.) Kickx.) is a devastating disease of certain two- and three-needle pines in the United States (3) and is especially serious in the Northeast. Japanese black pine has not been reported as a host for *S. sapinea*, whereas Austrian and Scots pines are highly susceptible. Increased susceptibility of pines to infection by *S. sapinea* resulting from drought or flooding has been noted

(1,2,9), yet experimental evidence of the effect of water stress is lacking. Water stress may influence plant disease by affecting the pathogen, host susceptibility, and/or the host-pathogen interaction (4,6,7). The effect of water deficits on the growth of *S. sapinea* within three pine species was investigated in this study.

MATERIALS AND METHODS

Three-year-old root-pruned Scots (*Pinus sylvestris* L.), Austrian (*P. nigra* Arnold), and Japanese black (*P. thunbergiana* Franco) pines were obtained from a nursery in central New Jersey in April 1981. Trees were potted in a loamy clay soil in 16-cm-diameter black plastic pots and grown in the greenhouse until the following February, when they were 40-60 cm tall and had produced several side branches.

Water was withheld from the trees to allow the soil to dry to three soil water potentials (SWP). Temperatures varied from 27 to 37 C in the greenhouse during

drying. Seventy-two trees selected for uniformity were used, eight trees of each species within each SWP. SWP was measured using calibrated gypsum blocks and moisture meters (Delmhorst Instruments, Boonton, NJ). A gypsum block was placed in the soil midway between the top and bottom of the pot and was not allowed to come in contact with the roots of the tree. Moisture meter readings were recorded until the average reading of all blocks within a group of 24 trees reached SWP of -0.2, -0.6 to -0.8, or -1.2 to -1.5 MPa.

After the soil had dried to the desired SWP, an 8-mm vertical incision was made along the main stem of each tree. A 3-mm disk of V-8 agar (V-8A) (200 ml of V-8 juice, 3 g of CaCO₃, 20 g of agar, and 1,000 ml of distilled water) containing mycelium from the margin of a 5-day-old *S. sapinea* culture isolated from Austrian pine was inserted in the incisions on half of the trees. V-8A disks without *S. sapinea* colonies were inserted in the remaining incisions as controls. The incisions were wrapped in moist cotton and covered with transparent tape. Trees were placed in a growth chamber and held at 22 ± 1 C, 95-100% relative humidity (RH), and incident light intensity of 75 lux (daylight and fluorescent) for 10 hr each day. These conditions were a modification of a method for controlling plant water potentials in disease susceptibility studies reported by Schoeneweiss (5). Trees were removed from the growth chamber after

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Table 1. Main effects of pine species and soil moisture potential on linear growth of *Sphaeropsis sapinea* in main stems (experiment 1)

Pine species ^a	Mean growth of <i>S. sapinea</i> (mm) ^b
Scots	38.7
Austrian	34.3
Japanese black	26.3
Water potential (MPa)^c	
-0.1	23.3
-0.6 to -0.8	32.8
-1.2 to -1.5	43.3

^a Data for all soil water potentials were combined.

^b Average number of serial 3-mm sections infected per stem.

^c Data for Scots, Austrian, and Japanese black pines were combined.

9 days.

Eighteen hours after removal from the growth chamber, stems were severed at soil line, pruned to leave only the main axis, and debarked. Sections 3 mm long were aseptically cut from each main stem from the middle of the incision toward each end and plated in series on V-8A. The entire length of the stem was sectioned, and the number of total sections per stem varied slightly according to the heights of individual trees. Plates were incubated at 24 C in the dark, and sections were checked for growth of *S. sapinea* after 48 hr.

A second experiment was run to corroborate data from the first experiment and to test if the low light intensity used in the first experiment had any effect on the host-pathogen interaction. Eighteen Scots pines selected from the tree lot used in the first experiment were allowed to dry to the same SWPs as before. Six trees

were included for each SWP; four were inoculated with *S. sapinea* in V-8A plugs and two received V-8A plugs without mycelium. The trees were placed in the same growth chamber at 95-100% RH, 22 ± 1 C, and 11,836 lux (incandescent and fluorescent) for 12 hr each day. Other aspects of the experiment were the same as in the first experiment.

RESULTS AND DISCUSSION

Areas of stem tissue within 2 mm of the incisions turned dark brown in both inoculated and control trees. In inoculated stems, growth of *S. sapinea* preceded the advance of sharply outlined, brown necrotic areas. No spreading necrosis occurred in control trees. Differences in growth of *S. sapinea* occurred among SWPs and tree species. Mean fungal growth of about 70, 98, and 130 mm occurred in stems with SWPs of -0.1, -0.6 to -0.8, and -1.2 to -1.5 MPa. Fungal growth increased in trees, regardless of their susceptibility to *S. sapinea*, with more negative SWP values (Table 1). Scots and Austrian pine supported more fungal growth than did Japanese black pine (Table 1). Although Japanese black pine is considered resistant to *S. sapinea*, the fungus grew as far as 72 mm from the inoculation point. Growth of *S. sapinea* occurred along the entire length of Scots pine stems at SWPs of -1.2 to -1.5 MPa and may have been greater if the stems had been longer.

In the second experiment, the response of Scots pine was generally the same as observed in the first experiment. Growth of *S. sapinea* in stem tissue again increased as the SWP values became more negative. Average numbers of serial 3-mm sections infected per tree were 19.0, 27.8, and 43.3 at SWPs of -0.1, -0.6 to -0.8, and -1.2 to -1.5 MPa, respectively.

S. sapinea was considered by some (1,2,8,9) to be a saprophyte or weak pathogen because of the ease with which injured tissue became infected but was later shown (3,8) to infect uninjured bud scales under the proper conditions. Its enhanced growth in droughted tissue may be due to an increase in the carbohydrate pool available to the pathogen or to a reduction in the rate of host defense reaction (6). Our experimental data support field observations (1,2,9) of increased incidence of tip blight caused by *S. sapinea* on droughted or unhealthy pine trees.

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