# DURATION OF WATER STRESS AFFECTS DEVELOPMENT OF SPHAEROPSIS CANKER ON SCOTS PINE

**by Janelle W. Johnson, Mark L. Gleason, Sharon K. Parker, Ellen B. Provin, Jeffery K. Iles, and Paula H. Flynn.**

**Abstract.** Saplings of *Pinus sylvestris* were subjected to drought stress periods and inoculated with *Sphaeropsis sapinea* in a greenhouse experiment. Six wk after inoculation, saplings that had low needle water potentials for 14 to 21 days after inoculation exhibited more dieback and developed significantly ($P < 0.05$) longer cankers than saplings that had equivalent water stress for 3 to 4 days after inoculation or unstressed saplings. The results indicate that drought stress increases susceptibility of Scots pine to Sphaeropsis canker and that canker growth can be suppressed by watering drought-stressed trees.

## Introduction

Cankers caused by the fungus *Sphaeropsis sapinea* (Fr.) Dyko and Sutton damage many conifers worldwide, especially pines planted outside their native ranges. Severe episodes of Sphaeropsis canker lead to crown wilting and top and branch dieback, resulting in extensive economic losses in plantations (2, 3, 7, 8, 10) and deformities in landscape trees (5). Many observations (e.g., 2, 7, 8, 9) have suggested that drought stress exacerbates damage from Sphaeropsis canker, but experimental evidence of this relationship is sparse (1, 4, 6). The only study documenting a statistically significant effect of drought stress on growth of Sphaeropsis canker was done on cypress (*Cupressus sempervirens* L.) (6).

In Iowa, Sphaeropsis canker occurs on many pine species and on white fir (*Abies concolor*) (5) but is most common on Scots pine. The present study was undertaken to determine if severity and duration of drought stress affect Sphaeropsis canker on Scots pine.

## Materials and Methods

In August 1991, 4-yr-old, nursery-grown Scots pine, with mean height of approximately 1 m, were potted in 26-liter liners containing a shredded bark medium (701 mix, W.R. Grace Co., St. Louis, MO) and grown in a greenhouse at 20 to 30° C. The experiment was conducted in June-August 1992. Experimental design was a randomized complete block with eight treatments and nine replications (individual saplings) per treatment. Treatments included a 2 x 2 factorial combination of water stress and timing of inoculation with *S. sapinea*. Water stress was imposed by withholding watering until needle water potential was either ≤-3.0 or ≤-4.5 MPa (1 MPa=10 bars). For treatments in which water stress preceded inoculation with *S. sapinea*, saplings were inoculated within one day after the water-potential thresholds were reached, and watering of pots was resumed 3 to 4 days later (Table 1). For treatments in which water stress was imposed after inoculation, watering was resumed on the same day that water-potential

**Table 1. Association of water stress in *Pinus sylvestris* saplings with occurrence of top dieback and mean canker length 6 wks. after inoculation with *Sphaeropsis sapinea*[a].**

| Water potential threshold[b] (-MPa) | Time water withheld before inoculation (days) | Time from inoculation to resumption of watering (days) | No. of trees with dead tops[c] | Mean canker length[d] (cm) |
|---|---|---|---|---|
| 3.0 | 7 | 3[e] | 0 | 7.7 |
| 4.5 | 10 | 4[e] | 2 | 9.7 |
| 3.0 | 0 | 14 | 2 | 22.9 |
| 4.5 | 0 | 21 | 7 | 25.4 |
| ≥1.0 (control) | -- | -- | 0 | 4.3 |

[a] Saplings in control treatments that were inoculated with sterile APDA disks developed no top dieback or cankers, so these treatments are omitted from the Table.
[b] Watering was resumed after mean needle water potential exceeded thresholds.
[c] Number of trees per treatment=9.
[d] LSD ($\alpha$=0.05, 40 df)=5.8.
[e] Inoculation occurred after needle water potential thresholds were exceeded.

thresholds were reached. A control treatment inoculated with *S. sapinea* was not subjected to withholding of water; instead, it was watered thoroughly every 2 to 3 days throughout the experiment. Saplings in three additional control treatments were inoculated with sterile disks of acidified potato-dextrose agar (APDA): 1) 3 to 4 days after needle water potentials were ≤ -4.5 MPa; 2) before watering was withheld to a needle water potential threshold of ≤ -4.5 MPa; or 3) without withholding watering.

Needle water potential was monitored with a pressure bomb (PMS Instrument Co., Corvallis, OR). Complete fascicle bundles were removed from 1-yr-old branches. After the fascicle sheath was removed, a bundle was inserted in a rubber stopper in the cap of the pressure bomb with the base of the fascicle facing upward. Gas pressure from a nitrogen tank was increased at the rate of -0.05 MPa/sec (K. Rane, Department of Botany and Plant Pathology, Purdue University, W. Lafayette, IN, *personal communication*). A pressure reading was made when a droplet of sap began to exude from the fascicle base. A dissecting microscope (30x magnification) was used to observe formation of the droplet. Readings were taken from 4 am to 5:30 am to ensure that stomates were closed and that plant moisture status was in equilibrium. Three saplings per treatment were monitored three times per wk to determine when water potential thresholds were reached. When mean needle water potential of this sample was less than or equal to the treatment thresholds, fascicles from the other six saplings in a treatment were measured.

An isolate of *S. sapinea* from a cankered Scots pine in Des Moines, IA was used to inoculate saplings. After a portion of the main stem 30 to 45 cm above the pot was wiped with 95% ethanol, a 5- x 5-mm segment of bark was removed with a flamed scalpel, exposing the outer sapwood. A 3-mm-diameter disc of APDA, either sterile (three control treatments) or containing the outer margin of an actively growing *S. sapinea* colony, was placed against the sapwood with the mycelium side of the disk facing the stem. The wound was covered with cotton, which was secured with adhesive tape and moistened with water (1).

Changes in foliage color, turgor, and branch dieback were noted weekly. Six weeks after inoculation, the main stem of each sapling was excised 30 cm above and below the inoculation site. Length and width of cankers surrounding the inoculation sites were then measured after scraping the outer bark with a scalpel to expose the canker margin. After debarking, a flamed scalpel was used to cut chips from the outer sapwood at 1-cm intervals from 20 cm above to 20 cm below the incubation site and the chips were cultured on APDA. Cultures were incubated at 20-25$^0$ C in continuous light and examined daily for fungal growth.

**Results**

Mean needle water potential declined to ≤-3.0 MPa within 10 to 14 days and ≤-4.5 MPa within 14 to 21 days after watering ceased (Table 1). For treatments in which water stress was imposed after inoculation, watering was withheld for 14 days (-3.0 MPa threshold) or 21 days (-4.5 MPa threshold) after inoculation. Needle water potential returned to ≥ -1.0 MPa within 24 hr after watering was resumed.

On saplings from which watering was withheld, needles gradually developed a dull color and became twisted, and branches began to droop. Drought symptoms were more pronounced on trees in the ≤-4.5-MPa-threshold treatments than in the ≤-3.0-MPa-threshold treatments. After inoculation with *S. sapinea*, droplets of resin exuded from wound sites, and cankers began to develop. No cankers were found on controls not inoculated with *S. sapinea*.

Beginning 3 wk after inoculation, some saplings died above the point of inoculation. In the ≤-4.5-MPa-threshold treatment in which watering was withheld for 21 days after inoculation, top dieback had occurred in seven of nine saplings within 6 wk after inoculation (Table 1). Two of nine saplings had dead tops in treatments in which needle water potential was held at ≤-4.5 MPa for 4 days after inoculation or brought to ≤-3.0 MPa at 14 days after inoculation. No top dieback occurred in the control treatments or in the treatment in which needle water-potential levels were held at ≤ -3.0 MPa for 3 days after inoculation.

*S. sapinea* was recovered consistently from sapwood samples within canker margins, except where dieback had occurred above the point of inoculation. Other fungi and actinomycetes recovered were, in order of decreasing frequency, *Penicillium* spp., *Pestalotia* spp., *Aureobasidium pullulans*, *Coniothyrium* spp., *Alternaria* spp., *Cladosporium* spp., and *Candida albicans*. Six wk after inoculation, mean canker length was significantly (P<0.001) greater for treatments in which water stress was imposed than for the inoculated control that received regular watering (Table 2). Saplings from which watering was withheld for 14 or 21 days after inoculation had significantly (P< 0.001) longer cankers than saplings from which watering was withheld for 3 or 4 days after inoculation. Water-potential threshold for the water-stressed treatments did not influence canker length significantly.

**Table 2. Orthogonal comparisons of canker lengths measured 6 wks. after inoculation with *Sphaeropsis sapinea*[a].**

| Comparison | F[b] |
|---|---|
| Unstressed control *vs.* water-stressed treatments[c] | 28.4*** |
| -3.0 MPa *vs.* -4.5 MPa watering threshold | 1.3 |
| 3-4 days *vs.* 14-21 days from inoculation to resumption of watering | 58.2*** |
| Watering threshold X duration of postinoculation, unwatered period (interaction) | 0.01 |

[a] General Linear Models procedure (SAS Inc., Goldsboro, NC).
[b] *** = P<0.001.
[c] Water-stressed treatments=mean of all -3.0 MPa and -4.5 MPa watering-threshold treatments.

## Discussion

The results are the first experimental evidence that water stress significantly increases the severity of Sphaeropsis canker on Scots pine. An earlier study (1) indicated a similar trend with decreasing needle water potential but did not test significance of observed differences in canker size. Our findings are consistent with field observations that drought stress exacerbates development of Sphaeropsis canker on other species of pine (e.g., 3, 7, 8) and on cypress (6).

The duration of water stress after inoculation influenced canker development. Saplings that were moderately (≤ -3.0 MPa) to severely (≤ -4.5 MPa) water-stressed were able to confine canker expansion when watering was resumed within a few days after inoculation. When water was withheld for 2 or 3 wk after inoculation, however, canker development proceeded much more rapidly, and top dieback was common after 3 wk of drought stress. Cankers were much smaller, and top dieback was absent, when saplings were watered regularly after inoculation. The results are consistent with the view that Sphaeropsis canker is an opportunistic disease whose severity is linked to environmental stress (4, 6). We showed that Scots pine can recover from severe drought stress and confine Sphaeropsis canker development. This finding suggests that regular watering can help to deter Sphaeropsis canker after infection has occurred.

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Department of Plant Pathology
Iowa State University
Ames, IA 50011

Department of Horticulture
Iowa State University
Ames, IA 50011

**Résumé.** Des jeunes *Pinus sylvestris* ont été soumis à des périodes de sécheresse et inoculés en serre expérimentale avec le Sphaeropsis sapinea. Six semaines après l'inoculation, les jeunes arbres, qui avaient des valeurs de potentiel en eau dans les aiguilles aussi basses que -3.0 à -4.5 Mpa durant 14 à 21 jours après l'inoculation, exhibaient un degré plus élevé de dépérissement sur le sommet et développaient aussi, de façon significative (P 0.05), des chancres plus gros que les jeunes arbres non stressés ou que ceux avec une période de stress en eau de 3 à 4 jours après l'inoculation. Les résultats indiquent que le stress de la sécheresse augmente la susceptibilité des pins sylvestres au chancre du Sphaeropsis et que la croissance du chancre peut être supprimée par l'arrosage des arbres affectés par le stress de la sécheresse.

**Zussammenfassung.** Die Heister von *Pinus sylvestris* wurden in einem Gewächschausexperiment periodisch der Trockenheit ausgesetzt und mit *Sphaeropsis sapinea* inokuliert. Sechs Wochen nach der Inokulation zeigten die Heister mit einem Blattwasserpotential won -3.0 oder -4.5 Mpa für einen Zeitraum von 14 bis 21 Tagen nach der Inokulation mehr Spitzensterben und entwickelten deutlich länger Baumkrebs ($p<0.05$) als entweder ungestresste Heister oder Heister mit Wasserstress über 3 bis 4 Tage nach der Inokulation. Die Resultate zeigen, daß Stress durch Trockenheit die Anfälligkeit der Gemeinen Kiefer für Sphaeropsis-Krebs erhöht und daß das Wachstum der Krebsgeschwüre durch Wässerung der trockenheitsgestressten Bäume unterdrückt werden kann.