Effects of Water Stress on the Development of Cambial Lesions Caused by *Cryphonectria cubensis* on *Eucalyptus grandis*

W. J. SWART and E. CONRADIE, Department of Plant Pathology, M. J. WINGFIELD, Department of Microbiology and Biochemistry, and W. B. VENTER, Department of Pasture Science, University of the Orange Free State, Bloemfontein 9300, South Africa

ABSTRACT

Swart, W. J., Conradie, E., Wingfield, M. J., and Venter, W. B. 1992. Effects of water stress on the development of cambial lesions caused by *Cryphonectria cubensis* on *Eucalyptus grandis*. Plant Dis. 76:744-746.

The restriction of *Cryphonectria cubensis* to high-rainfall areas in South Africa has led to speculation regarding the influence of soil moisture on disease severity. A study was, therefore, conducted to determine whether a relationship exists between the development of Cryphonectria canker and water stress. Techniques involving axenic culture of the pathogen and artificial inoculation of 1-yr-old potted *Eucalyptus grandis* plants were employed. In culture, a significant negative correlation ($r = 0.993$; $P < 0.01$) was found between the growth of *C. cubensis* and osmotic stress induced by KCl. Pressure bomb assessment of the water potentials of inoculated plants indicated that drought-stressed plants (−2.5 MPa) developed smaller cambial lesions than nonstressed plants (−1.0 MPa). These results are in contrast with numerous reports on other canker pathogens in which drought stress is associated with increased growth of the pathogen.

Cryphonectria canker of *Eucalyptus* spp., caused by the fungus *Cryphonectria cubensis* (Bruner) C. S. Hodges, occurs within 30° north and south of the equator (6). The distribution of Cryphonectria canker is probably determined by humid conditions needed for the growth and spread of the pathogen. The disease is favored by high rainfall (2,000 - 2,400 mm/yr), high elevation, and temperatures above 23 C (8,18). In Brazil, *C. cubensis* causes heavy losses in areas where high rainfall occurs throughout the year, and temperatures average 23 C or higher (9). In cooler or drier areas of Brazil, infection rates are much lower as is the extent of canker development (9). In other parts of the world where *C. cubensis* occurs, climate also seems to determine the severity of the disease (17).

In South Africa, the occurrence of Cryphonectria canker appears to be limited to the warmer parts of the country that have relatively high rainfall (25). This has led to speculation that low rainfall in other areas might inhibit the ability of *C. cubensis* to infect and colonize host tissue. Cankers caused by *Thyronectria austroamericana* (Speg.) Seeler on honey locust (*Gleditsia triacanthos* L.) are smaller on drought-stressed than on well-watered trees (11). In contrast, physiological stress and drought are known as major predisposing factors in many other canker diseases of trees (1-3,7,10,11). There are, however, no studies of the relationship between drought stress and development of Cryphonectria canker on *Eucalyptus* spp.

The objectives of this study were to determine whether a relationship exists between the in vivo growth of *C. cubensis* and the availability of soil moisture. The in vitro growth of the pathogen when exposed to osmotic stress in an axenic culture system was also studied.

MATERIALS AND METHODS

In vitro osmotic stress. Growth studies were performed on a medium (MYP) consisting of Difco malt extract (7 g), yeast extract (0.5 g), peptone (1 g), Difco Bacto Agar (15 g), and 1 L of water. The osmotic potential was adjusted by adding KCl at selected molal concentrations. The osmotic potentials of the treatments before inoculation were −0.5, −1.0, −1.5, −2.0, −2.5, −3.0, −3.5, −4.0, and −4.5 MPa, respectively. MYP lacking KCl has an osmotic potential of −0.14 MPa (12). The water potential of each treatment was determined using the following equation (5): $\psi_T = \psi_{MYP} + \psi_{KCl}$, where ψ_T = total water potential, ψ_{MYP} = water potential of MYP, ψ_{KCl} = water potential of KCl (15), $\psi_{KCl} = \log a_w / 3.199836 \times 10^{-4}$, where a_w = water activity. The a_w values for 0.1-1.0 molal KCl of Robinson and Stokes (16) were used.

Agar cultures at different water potentials were initiated by transferring 5-mm agar plugs cut from the margin of an actively growing colony of *C. cubensis* on potato-dextrose agar (PDA) to 90-mm petri plates. Each water

Accepted for publication 2 January 1992.

potential was replicated four times. Plates were incubated at 30 C, and growth was determined by measuring the colony diameter along two perpendicular lines after 4 days. The experiment was arranged as a randomized complete block design and conducted twice. A two-way analysis of variance was performed on the pooled data, and Tukey's HSD was applied for comparison of means. An analysis of the relationship between colony diameter and osmotic concentration was performed.

Glasshouse inoculations. One-year-old plants of a single clone of *E. grandis* A. W. Hill ex Maiden were grown in 1.5-L containers until root systems were well established. Plants were incubated in a glasshouse under conditions of 23–25 C, 70–90% relative humidity, and low light intensity. Under these semi-equilibrium conditions plant water potentials remained relatively stable (20,23). A group of plants was then subjected to drought stress by withholding water for 7–9 days. Water potentials of these plants were then determined in a pressure bomb as described by Schoeneweiss (20) and ranged between −3.5 and −4.0 MPa. A further group was stressed by withholding water (4–6 days) until the water potentials were between −2.0 and −2.5 MPa. Although a pressure bomb does not measure the osmotic component of the total xylem water potential, this component is small enough to be considered negligible at the low water potentials used in disease studies (20). Following the measurement of stress levels in each group, plants were watered with 400 ml of water to alleviate stress. Water was again withheld from both groups of plants until they reached the appropriate water potentials. A third group of plants was watered daily with approximately 200 ml of water. The three different watering regimes were maintained over a period of 5 wk.

Inoculation of plants with *C. cubensis* was performed 2 wk after the watering regimes had been implemented. Inoculum of *C. cubensis* was prepared by culturing the fungus in petri dishes on PDA overlaid with sterile gauze strips (10 × 50 mm) for 10 days. All plants were wounded by lightly scraping off a length of bark (3 × 10 mm) from each stem. An equal number of plants in each group was inoculated by wrapping a piece of gauze, colonized by *C. cubensis*, around each wound. Sterile gauze strips were wrapped around an equal number of control plants in each group. Parafilm (American National Can) was then wrapped around all wounds. Three weeks after inoculation, gauze strips were removed from all plants, and the total length of cambial discoloration on each stem was measured following complete removal of the bark with a scalpel. Isolations were made from all lesions to verify the presence of *C. cubensis*. The trial was arranged as a completely randomized design with 15 seedlings per treatment, and a two-way analysis of variance was performed on the data. Tukey's HSD procedure for comparison of means was applied where analyses of variance showed significant variation.

A second similar experiment was conducted in the glasshouse with 1-yr-old plants from each of three *E. grandis* clones. The plants were transferred to pots containing a growing medium composed of 40 g of horticultural grade vermiculite, 400 g of soil, and 600 g of sand in 1.5-L pots. Fifteen plants from each clone were water-stressed as follows. Four weeks prior to inoculation, plants were each given 400 ml of water and then allowed to dry for 7 days before the procedure was repeated. A second group of plants from each clone was given 150 ml of water every second day during the first 2 wk. At the end of the 2-wk period, water potential measurements were taken on all stressed and nonstressed plants by placing one mature leaf from each plant in a pressure bomb. The mean water potentials for stressed and nonstressed plants were −2.5 and −1.0 MPa, respectively. The total weight of each pot was also recorded at this point. Stress levels were then maintained for a further 2 wk by adding only enough water every day to maintain pot weights. Plants were wounded as previously described and inoculated using the gauze method. Control plants were treated as above. A water potential measurement was determined at 3, 7, and 10 days after inoculation, and the trial was terminated 14 days after inoculation. The surrounding bark was then removed, and the length of cambial discoloration measured. Isolations were made from discolored tissue to verify the presence of *C. cubensis*. The trial was arranged as a completely randomized design with 15 seedlings per treatment, and the experiment was conducted twice. A two-way analysis of variance was performed on the pooled data, and Tukey's HSD procedure was applied for comparison of means.

RESULTS AND DISCUSSION

In vitro osmotic stress. Increased KCl significantly reduced the growth of *C. cubensis* in agar culture. Growth varied significantly ($P < 0.01$) between each KCl concentration, and a significant linear relationship ($r = 0.993$; 7 df; $P < 0.01$) was found between KCl concentration and mean colony diameter of the two trials (Fig. 1). The method used to control in vitro osmotic stress is versatile and easy to apply, and the possibility of toxic effects on *C. cubensis* was taken into consideration. Previous studies (4,11,14,24) indicate that the general results obtained with KCl are largely attributable to osmotic stress, not to toxic effects, and are representative of results obtained with other commonly used osmoticants.

Glasshouse inoculations. In both glasshouse experiments, all plants inoculated with *C. cubensis* displayed cambial lesions. Control plants displayed no cambial discoloration. Although the mean lesion length (45.8 mm) for the most highly stressed plants (7–9 days between watering, mean water potential = −3.6 MPa) in the first experiment was smaller than that for the 4- to 6-day watering interval ($\psi = -2.3$, lesion length = 52.2) or the 1-day interval ($\psi > -1.0$, lesion length = 49.6 mm) treatments, differences were not statistically significant ($P = 0.05$). In the second experiment, however, cambial lesions were significantly shorter ($P < 0.01$) on stressed plants than on nonstressed plants (Fig. 2). There was also a significant interaction ($P < 0.01$) between clones and stress levels.

The discrepancy between the first and second experiments with regard to the effect of water stress on length of cambial lesions may be ascribed to the different techniques used to control water stress in each case. Techniques that simply vary the period between watering, as in the case of the first experiment, have limited accuracy. This is because plants are subjected to varying levels of soil water content between saturation capacity and the permanent wilting percentage. This exposes plants to an average water stress over an extended period, which may have little relevance as to the actual physiological status of plant tissues and its effect on disease susceptibility (19–21).

The results of this study indicate that the colonization of young *Eucalyptus* plants by *C. cubensis* following artificial inoculation is inhibited by drought stress. The reduced growth of *C. cubensis* in axenic culture at low osmotic potentials substantiated the inoculation results. The results agree with work on Thyronectria bark canker on honey locusts in which drought-stressed seedlings had smaller cankers than nonstressed seedlings (11). They are, however, in contrast to numer-

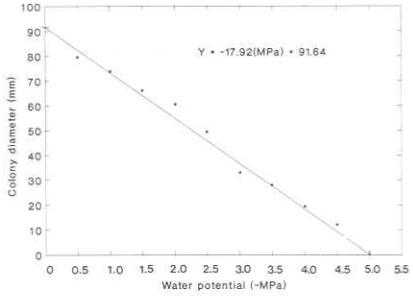

Fig. 1. Effect of water potential on colony diameter after 4 days of *Cryphonectria cubensis* on MYP (Malt extract, yeast extract, and peptone) amended with varying concentrations of KCl to give varying water potentials.

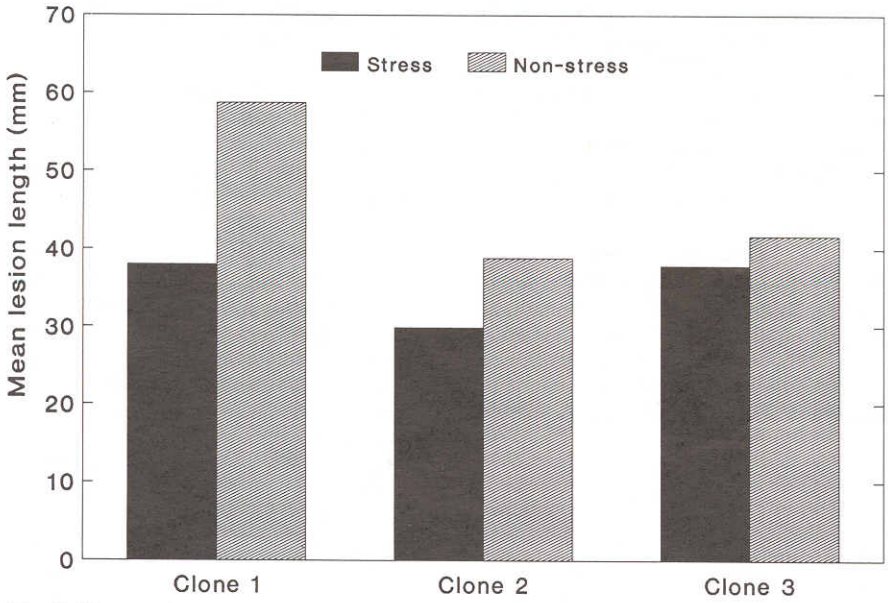

Fig. 2. Mean lesion length on three *Eucalyptus grandis* clones subjected to water stress and inoculated with *Cryphonectria cubensis* in the glasshouse.

ous reports associating water stress in plants with increased susceptibility to canker pathogens (2,3,13,22). The interaction between clones and stress levels in the second experiment suggests that some *E. grandis* clones may be less susceptible to Cryphonectria canker by virtue of their ability to withstand drought. It therefore seems apparent that much basic information on the effects of drought stress can be obtained by working with genetically uniform plants such as clones.

Cryphonectria canker is favored by high rainfall (2,000–2,400 mm/yr) and temperatures above 23 C (8,18). In Brazil, *C. cubensis* causes far heavier losses in areas where high rainfall occurs throughout the year than in the cooler, drier areas of the country (9). The results of this study, in which disease was reduced by stress, are therefore consistent with the restriction of the disease to high rainfall areas in South Africa (6).

The results presented in this study are consistent with the known epidemiology of *C. cubensis*. However, results obtained under such rigidly controlled conditions as in the greenhouse studies should be viewed with some circumspection. Such results may not be consistent with those found under field conditions, which are characterized by interacting environmental factors that are cyclical and varied (19). It is, therefore, important that field studies be initiated to further investigate the influence of drought stress on Cryphonectria canker of *Eucalyptus* in South Africa.

LITERATURE CITED

1. Appel, D. N., and Stipes, R. J. 1984. Canker expansion on water-stressed pin oaks colonized by *Endothia gyrosa*. Plant Dis. 68:851-853.
2. Bagga, D. K., and Smalley, E. B. 1974. The development of hypoxylon canker of *Populus tremuloides*: Role of interacting environmental factors. Phytopathology 64:658-662.
3. Bertrand, P. F., English, H., Uriu, K., and Schick, J. 1976. Late season water deficit and development of Cytospora canker in French prune. Phytopathology 66:1318-1320.
4. Boddy, L. 1983. Effect of temperature and water potential on growth rate of wood-rotting basidiomycetes. Trans. Br. Mycol. Soc. 80:141-149.
5. Boyer, J. S. 1969. Measurements of the water status of plants. Annu. Rev. Plant Physiol. 20:351-364.
6. Conradie, E., Swart, W. J., and Wingfield, M. J. 1990. Cryphonectria canker of *Eucalyptus*, an important disease in plantation forestry in South Africa. S. Afr. For. J. 152:43-49.
7. Crist, C. R., and Schoeneweiss, D. F. 1975. The influence of controlled stresses on susceptibility of European white birch stems to attack by *Botryosphaeria dothidea*. Phytopathology 65:369-373.
8. Florence, E. J. M., Sharma, J. K., and Mohanan, C. 1986. A stem canker disease of *Eucalyptus* caused by *Cryphonectria cubensis* in Kerala. Kerala For. Res. Inst. Sci. Pap. 66:384-387.
9. Hodges, C. S., Geary, T. F., and Cordell, C. E. 1979. The occurrence of *Diaporthe cubensis* on *Eucalyptus* in Florida, Hawaii, and Puerto Rico. Plant Dis. Rep. 63:216-220.
10. Hunter, P. P., Griffin, G. J., and Stipes, R. J. 1976. The influence of osmotic water potential on linear growth of *Endothia* species. Phytopathology 66:1418-1421.
11. Jacobi, W. R., and Riffle, J. W. 1989. Effects of water stress on Thyronectria canker of honeylocusts. Phytopathology 79:1333-1337.
12. Koske, R. E., and Tessier, B. 1986. Growth of some wood and litter-decay basidiomycetes at reduced water potential. Trans. Br. Mycol. Soc. 86:156-158.
13. Madar, Z., Solel, Z., and Kimchi, M. 1989. Effect of water stress in cypress on the development of cankers caused by *Diplodia pinea* f. sp. *cupressi* and *Seiridium cardinale*. Plant Dis. 73:484-486.
14. Malajczuk, N., and Theodorou, C. 1979. Influence of water potential on growth and cultural characteristics of *Phytophthora cinnamoni*. Trans. Br. Mycol. Soc. 72:15-18.
15. Prior, B. A. 1979. Measurement of water activity in foods: A review. J. Food Prot. 42:668-674.
16. Robinson, R. A., and Stokes, R. H. 1959. The measurement of chemical potentials. Pages 174-222 in: Electrolyte Solutions. R. A. Robinson and R. H. Stokes, eds. Butterworths Scientific Publications, London. 559 pp.
17. Sharma, J. R., Mohanan, C., and Florence, E. J. M. 1985. Disease survey in nurseries and plantations of forest tree species grown in Kerala. Kerala For. Res. Inst. Res. Rep. 36.
18. Sharma, J. R., Mohanan, C., and Florence, E. J. M. 1985. Occurrence of Cryphonectria canker disease of *Eucalyptus* in Kerala, India. Ann. Appl. Biol. 106:265-276.
19. Schoeneweiss, D. F. 1975. Predisposition, stress and plant disease. Annu. Rev. Phytopathol. 13:193-211.
20. Schoeneweiss, D. F. 1975. A method for controlling plant water potentials for studies on the influence of water stress on disease susceptibility. Can. J. Bot. 53:647-652.
21. Schoeneweiss, D. F. 1978. Water stress as a predisposing factor in plant disease. Pages 61-99 in: Water deficits and plant growth. Vol 5. T. T. Kozlowski, ed. Academic Press, New York. 322 pp.
22. Schoeneweiss, D. F. 1983. Drought predisposition to Cytospora canker in blue spruce. Plant Dis. 67:383-385.
23. Slatyer, R. O. 1967. Environmental aspects of plant-water relationships. Pages 27-64 in: Plant-Water Relationships. R. O. Slatyer, ed. Academic Press, New York. 366 pp.
24. Sommers, L. E., Harris, R. F., Dalton, F. N., and Gardner, W. R. 1970. Water potential relations of three root-infecting *Phytophthora* species. Phytopathology 60:932-934.
25. Wingfield, M. J., Swart, W. J., and Abear, B. J. 1989. First record of Cryphonectria canker of *Eucalyptus* in South Africa. Phytophylactica 21:311-313.