HORTSCIENCE 27(5):414-416. 1992.

Short- and Long-term Heat Stress Effects on Phytophthora Root Rot of *Hibiscus*

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Additional index words. *Phytophthora parasitica*, root infection, predisposition

Abstract. **Roots of hydroponically grown *Hibiscus Rosa-sinesis* L. cuttings were exposed to 22, 30, 40, or 50C for 20 minutes, after which they were inoculated with zoospores of *Phytophthora parasitica* Dastur. Visual assessment of root discoloration and culturing of randomly selected root pieces 10 to 13 days after treatment showed that roots exposed to 40 or 50C had a significantly higher incidence of infection than those exposed to 20 or 30C. Plants were also grown in pots containing University of California (UC) mix or washed, graded sand and exposed to solar radiation for 1 day or 3 weeks, respectively. Root systems of plants in direct sunlight heated to 52C, while roots of shaded plants heated to 40C. Assessment of infection severity was done visually or by means of a *Phytophthora*- specific antibody probe. In all experiments, infection severity was highest in sun-exposed plants and was insignificant to moderate in shaded plants.**

Phytophthora root rots are an important group of diseases affecting container-grown plants. Research has shown that these diseases can have severe effects on plants exposed to environmental stresses resulting from extremes in soil water status (Blaker and MacDonald, 1981; Duniway, 1977; Wilcox and Mircetich, 1985), oxygen deficiency (Filmer et al., 1986; Heritage and Duniway, 1985), and salinity (MacDonald, 1984; Swiecki and MacDonald, 1988). Another stress that can affect roots of container-grown plants is heat, caused by solar radiation on the soil surface and/or on exposed container walls. While root-zone temperature has been studied in relation to disease, its effects generally have been attributed to growth activity of pathogens (Hine et al., 1964; Patil and Young, 1960). Relatively few studies have dealt with heat as a stress factor that may predispose roots to pathogenic attack.

In experiments with soybean *[Glycine max* (L.) Merr.] Chamberlain and Gerdemann (1966) found that roots of resistant cultivars became susceptible to *Phytophthora megasperma* var. *sojae* (Hildebrand) after a 1-h immersion in a 44C water bath. Exposure to 50C for as little as 2 min caused irreversible susceptibility (Chamberlain, 1970). Similarly, MacDonald (1991) showed that temperatures of 40C or higher predisposed *Chrysanthemum [Dendranthema grandiflorum* (Ramat.) Kitamura] roots to severe *Phytophthora cryptogea* Pethyb. & Laff. infection. These experiments differed from those of Chamberlain and Gerdemann (1966) in the use of plants grown in potting medium and solution culture but still involved single-pulse stress events (MacDonald, 1991). In nurseries, plants may be exposed to cycles of heating and cooling that continue for months. The effects of prolonged heating on root disease are still largely unknown. Knowledge of how soil temperature influences disease severity is critical in developing nonchemical management strategies to reduce the incidence and severity of phytophthora root rot. The purpose of this research was to determine the role of heat stress in phytophthora root rot severity on Chinese hibiscus.

Terminal stem cuttings of the 'Kona' hibiscus were rooted by dipping in 33 mM (8000 ppm) potassium salt of indole-3-butanoic acid (K-IBA) for 30 to 45 sec, air-drying for 5 min, and then suspending the cut ends in aero-hydroponic units half-filled with distilled water (Soffer and Burger, 1989). Cuttings with roots 1 to 2 cm long were used to initiate all experiments. All inoculations used an isolate of *P. parasitica* originally recovered from a diseased hibiscus plant collected from a southern California nursery. Zoospore inoculum was prepared using standard methods (Blaker and MacDonald, 1981; MacDonald, 1991; Swiecki and MacDonald, 1988), and spore concentrations were determined with a hemacytometer.

To determine the impact of short-term heat exposure on disease severity, rooted cuttings were transferred to 2-liter ceramic crocks containing aerated, half-strength Hoagland's Solution no. 2 (Hoagland and Arnon, 1950) amended with 2.5 meq NH_4NO_3/liter. Sixteen crocks, each containing three plants, were prepared. Plants were grown in hydroponic culture for 9 days before exposure to heat stress. To expose roots to various temperatures, 600-ml beakers containing 500 ml of nutrient solution were placed in heated water baths and allowed to equilibrate to 22 (room temperature), 30, 40, or 50C. Twelve plants were exposed to each temperature by immersing their roots in the beakers of aerated Hoagland's solution for 20 min. Following removal from the water baths, six plants from each temperature treatment were inoculated by immersing their roots for 4 h in beakers containing 1.44×10^6 zoospores diluted in 500 ml of a 1% (10 g soil/liter distilled water) soil extract (MacDonald, 1982). Roots of noninoculated plants were immersed in beakers containing 500 ml distilled water. During inoculation, care was taken to assure that the crowns were not submerged in the inoculum suspension. After inoculation, plants were returned to the ceramic crocks. The half-strength Hoagland's solution in the crocks was changed at the time of inoculation and topped-off as required for the remainder of the growth period.

Plants were harvested 10 and 13 days after inoculation for Expts. 1 and 2, respectively, and evaluated for disease severity using two methods. The percent infection in each root system was assessed using a pretransformed scale (Little and Hills, 1978), where 0 = 0%, 1 = 1% to 15%, 2 = 16% to 50%, 3 = 51% to 85%, and 4 = >85% of the root system discolored. After the plants were rated visually, 20 randomly selected root pieces from each plant were cultured onto a primaricin, ampicillin, rifampicin, penicillin

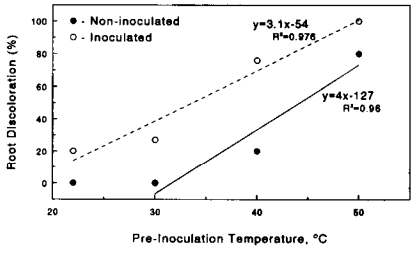

Fig. 1. Linear regression analysis showing the relationship between percent overall root discoloration and preinoculation temperature exposures for noninoculated and inoculated *Hibiscus* plants growing in aerated solution culture.

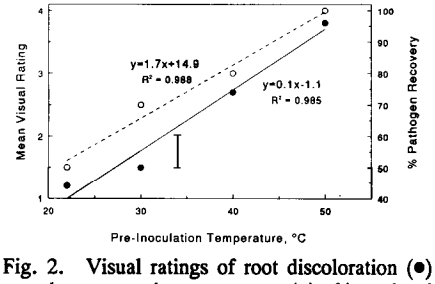

Fig. 2. Visual ratings of root discoloration (●) and percent pathogen recovery (○) of inoculated *Hibiscus* plants after various preinoculation temperature exposures. Linear regression response lines are shown. Mean visual rating differed significantly ($P = 0.05$) between all inoculated plants and the non-heat-injured, noninoculated plants. The vertical bar represents the LSD = 0.53 for the mean visual rating data.

Received for publication 6 Aug. 1991. Accepted for publication 9 Dec. 1991. We acknowledge the technical assistance of P.A. Kiehl. This work was supported, in part, by the Elvenia J. Slosson Fund for Ornamental Horticulture. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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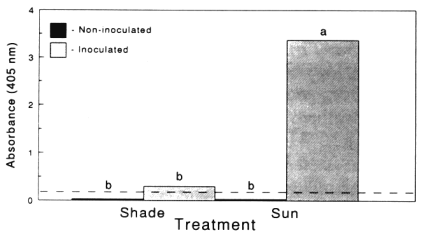

Fig. 3. ELISA absorbance results from *Hibiscus* roots from inoculated and noninoculated plants kept in the shade or sun. Bars with the same letter above them are not significantly different at $P = 0.05$ using Scheffe's mean separation procedure. The horizontal dashed line indicates the positive/negative threshold for the ELISA test determined by the mean absorbance of the shaded, noninoculated plants plus three SD.

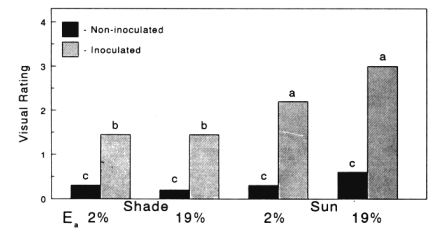

Fig. 4. Visual rating of root system discoloration of inoculated and noninoculated *Hibiscus* plants grown in the shade or sun in two different container media having air-filled porosities (E_a) of 2% and 19%. Bars with the same letter above them are not significantly different at $P = 0.05$ using Scheffe's mean separation procedure.

(PARP) selective agar medium to determine the percentage of segments colonized by *P. parasitica.* Tissue from necrotic crowns also was cultured to verify infection. In addition, the percentage of primary roots (main roots originating from the plant crown) with lesions was determined, and randomly selected lesions were plated onto PARP to verify the presence of *P. parasitica.* The entire experiment was repeated.

In other short-term heat experiments conducted in late spring, rooted cuttings were transplanted into 12.7 × 12.7 cm (diameter/height) black pots containing 900 cm^3 UC mix (1 peat : 1 redwood sawdust : 1 sand, by volume). Twenty-four plants were placed singly and off-centered (≈2.5 cm from the container wall) in pots. They were then grown in a greenhouse with no supplemental lighting and day/night cycles of 21/15.5C; they were irrigated daily with an amended, half-strength Hoagland's solution no. 2. After 15 days, all plants were moved to an outdoor nursery at ≈10:00 AM. The pots were oriented with the planted side facing west to maximize late afternoon sun exposure on the portion of the container wall nearest the roots. Half the plants were placed under 72% shade cloth while the other half received full sun.

Root-zone temperatures in several sun-exposed and shaded pots were monitored every 30 min using thermocouples (inserted at the time plants were placed in the field) attached to a data logger (Easylogger, Omnidata, Logan, Utah). After the maximum temperature for the day was achieved (6:00 PM), the plants were moved back into the greenhouse, placed in tubs containing distilled water to a level 6 cm below that of the pot soil surface. Half the plants were then inoculated by adding 5 × 10^5 or 2 × 10^6 zoospores per plant (Expts. 1 and 2, respectively) in 50 ml distilled water. Noninoculated plants were treated the same, except distilled water was added in the place of inoculum. The elevated water levels in the pots prevented inoculum from flowing out the bottom of the pots.

Plants were harvested 8 days (Expt. 1) and 10 days (Expt. 2) after inoculation. Plants were removed from the containers, and the roots nearest the sun-exposed wall were excised by cutting a 2.5-cm-thick, full-depth slice from the root ball. Root samples were placed on sieves and washed to remove potting medium. Relative infection severity for each plant was assessed by removing a 0.5 g subsample of washed roots and assaying for *P. parasitica* using a commercial enzyme-linked immunosorbant assay (ELISA) test (Agri-Diagnostics, Cinnaminson, N.J.). These tests detect antigen produced by *Phytophthora* spp. and can be used to estimate infection severity (MacDonald et al., 1990).

In long-term exposure experiments, rooted cuttings were transplanted into 12.7 × 12.7 cm (diameter/height) black containers filled with one of two sands. The sands (Lonestar Sand, Sacramento, Calif.) were graded types (Sands 1C and 3) having air-filled porosities (E_a) at container capacity of 2% and 19%, respectively. A total of 72 plants were potted for each experiment; half were potted into Sand 1C and half into Sand 3. After potting, the plants were grown for 10 to 16 days in a greenhouse with no supplemental lighting and day/night cycles of 21/15.5C. Plants were irrigated daily with an amended, half-strength Hoagland's solution no. 2 and were shaded under cheesecloth for the first 7 days following potting. After establishment, plants were placed in tubs of distilled water and inoculated by evenly distributing 2.5 × 10^6 zoospores in 50 ml soil extract over the sand surface. Noninoculated plants were treated the same as inoculated plants, except distilled water was added in the place of inoculum. Half the plants in each sand treatment were inoculated, with the balance remaining as noninoculated controls. Three hours after inoculation, plants were removed from the tubs and transferred to an outdoor nursery bed. There, the plants were irrigated using a computer-controlled irrigation system (Lieth and Burger, 1989) that applied ≈100 ml to each container every 30 min to keep the containers at or near container capacity. The moisture content at container capacity for sands 1C and 3 were 34% and 37%, respectively. Half the inoculated and noninoculated plants from each of the two sands were placed under 72% shade cloth while the other half received full sun. Root-zone temperatures were recorded every half-hour by means of buried thermocouples attached to an Omnidata data logger.

Three weeks after inoculation, plants were removed from the containers and their roots washed free of sand under running tap water. Disease severity in washed roots was assessed visually using the rating scale described above. Roots and shoots were separated by cutting stems at the soil line. Root fresh weights were determined, after which roots and shoots were dried for 10 days in a 75C oven, and dry weights determined. In Expt. 1, a 0.5-g subsample of roots was excised after fresh-weight measurement and assayed for *P. parasitica* antigen using an ELISA test. The entire experiment was done twice.

In the hydroponic experiments, disease symptoms were visible in all inoculated treatments within 10 days of inoculation. Visual examination indicated a positive relationship between degree of heat stress and severity of root infection. In the absence of *Phytophthora,* temperatures ≤ 30C caused no visible root discoloration (Fig. 1). Inoculated plants at these same temperatures developed discoloration on 20% to 30% of their root systems. Plants exposed to 40C wilted, and ≈20% of the root systems of noninoculated plants turned brown (Fig. 1) with necrotic root tips. Among inoculated plants at 40C, 75% developed root discoloration and 83% of the plants had confirmed crown infections. The 50C treatment caused substantial, direct root injury, with 80% of the roots of noninoculated plants being discolored (Fig. 1). Nearly 100% of the roots from inoculated plants exposed to the same temperature were discolored (Fig. 1), and fungal colonization of the crowns and lower stems of all plants was confirmed. All plants in the 50C treatment groups had wilted 1 week after treatment. The reliability of the visual assessment of root infection from inoculated plants was confirmed in culture-plating experiments. As the temperature to which the roots were exposed before inoculation increased, so did both the disease rating and the percent of root segments from which the pathogen could be recovered on selective medium (Fig. 2).

In the short-term nursery bed experiments, average root zone temperatures recorded for containers in full sun increased from a low of 34C (at 10:00 AM) to a high of 52C (at 6:00 PM); those in the shade increased from 28 to 40C over the same period. Five days after inoculation, all of the sun-exposed, inoculated plants were severely wilted, while none of the shaded, inoculated plants were wilted. In addition, all of the sun-exposed, inoculated plants developed crown infections, while there was no evidence of crown infection on the shaded, inoculated plants. ELISA analysis of root samples showed high levels of *Phytophthora* antigen in roots of sun-exposed, inoculated plants and relatively low levels of antigen (indicated by absorbance readings at 405 nm) in roots of shaded, inoculated plants (Fig. 3).

In the long-term nursery bed experiments, the sun-exposed, inoculated plants had the highest mean disease ratings in both the 2% E_a and 19% E_a sands (Fig. 4). The degree of root necrosis in the inoculated, shaded plants grown in the 2% E_a or 19% E_a sands was greater than in the noninoculated control plants. Significant differences in root fresh

or dry weights, or top dry weights, did not develop within the time limit of this experiment.

These experiments did not reveal a consistent temperature threshold that predisposes hibiscus roots to infection. The hydroponic experiments indicated that temperatures >30C can greatly increase infection severity. However, outdoor experiments indicated temperatures needed to exceed 40C. Root-zone temperatures >40C, which can last for several hours, have been recorded in container-grown plants during the summer (MacDonald, 1991). The temperature threshold of porous, solid media may be higher than that of liquid media because of the more gradual temperature increase associated with solar heating and better aeration in the roots.

The outdoor experiments showed that root-zone temperatures and phytophthora root rot severity could be reduced with shading. The E_a of the sand medium had no significant effect on disease in these experiments with hibiscus; however, E_a has been shown to be a factor in other plants (Filmer et al., 1986). The lack of E_a effect here may be due to differences in evaporative water loss. The shade cloth decreased the rate of evapotranspirational loss from the shaded plants relative to those in full sun. As a result, the sun-exposed plants most likely fell below container capacity many times throughout the course of the experiment, possibly affecting secondary inoculum production.

The experiments clearly show that high soil temperature can increase the severity of phytophthora root rot in container-grown *Hibiscus*. Management practices that reduce root-zone heating (e.g., shading or mulching around pots to prevent direct solar radiation) may help reduce the possibility or severity of root infection.

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