Delimitation of Lesions of Fusarium Hypocotyl Rot of Pine by Soil Microsite Environmental Determinants

K. H. Brownell and R. W. Schneider

Stauffer Chemical Co., P.O. Box 760, Mountain View, CA 94040, and Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge 70803.
Accepted for publication 12 June 1984.

ABSTRACT

Brownell, K. H., and Schneider, R. W. 1985. Delimitation of lesions of Fusarium hypocotyl rot of pine by soil microsite environmental determinants. Phytopathology 75:58-60.

Moisture and temperature in the top 3 cm of soil were tested for effects on the development of Fusarium hypocotyl rot of *Pinus lambertiana*, lesions of which occurred at a mean soil depth of 1.7 cm. Several factors were found to be involved in the depth restriction of lesion formation. Soil water potential and temperature were more favorable for fungal development with increasing soil depth, which would increase the probability of lesions occurring at greater depths. However, hypocotyl tissue was predisposed to localized infection by the higher temperatures that occurred in the top 3 cm. This predisposition did not persist after the heat was discontinued. Plant water stress of the magnitude found in the field did not predispose plants. This knowledge has been used to control the disease by manipulation of the soil environment at the microsite by earlier planting dates and partial shading.

Additional key words: *Fusarium oxysporum*, sugar pine.

Fusarium hypocotyl rot causes severe losses in sugar pine (*Pinus lambertiana* Dougl.) in forest nurseries (2). The pathogen, *Fusarium oxysporum* (Schlecht.) em. Snyd. & Hans., causes lesions between 1 and 3 cm below the soil line (2) with 82% of the lesions restricted to within 0.5 cm of the mean of 1.7 cm. The reason for this restricted site of lesion formation was unknown. Earlier work showed that root tissue was resistant to hypocotyl rot; however, observations that lesions occurred less frequently in the distal hypocotyl tissue indicated that other factors may influence the deeper limit of lesion formation.

The surface of agricultural soils undergoes radical diurnal changes in both temperature and moisture. During the day, solar radiation can heat surface soil to high temperatures; at night, thermal radiation from the surface causes a rapid decline in temperature (1). Following irrigation or precipitation, the surface soil becomes saturated, and, because of solar radiation and wind, quickly dries to extremely low water potentials. These rapid fluctuations in temperature and moisture can greatly affect both plants and microorganisms in this stratum.

This investigation concerns the dynamic surface soil microenvironment and its effect on a conifer seedling disease, Fusarium hypocotyl rot, which occurs in this stratum.

MATERIALS AND METHODS

To determine the extent of diurnal temperature and moisture fluctuations in the surface soil, these variables were recorded during a period of 4 days at six locations in a forest nursery at Magalia, CA. Weather conditions during this time were representative of those occurring when the disease is most severe (2). Temperature was recorded hourly to a depth of 5 cm at 6-mm intervals in undisturbed soil. This was accomplished by inserting thermocouple probes 5 cm into the vertical face of a small hole that was then filled. The surface (0 mm) thermocouple was placed on the soil surface and lightly covered with fine soil. Soil water content was determined from soil samples taken to a depth of 5 cm at 6-mm intervals at three locations. Samples were collected before sunrise, after sunset, and 0.5 hr before and after watering for two 2-day periods. Samples were dried at 105 C for 24 hr for determination of percent moisture. The moisture release curve (relationship between percent moisture content and water potential) was established by using a pressure plate extractor (−0.2 to −15 bars) and an isopiestic thermocouple psychrometer (−15 to −150 bars).

Total water potential (Ψ) of 10 randomly selected seedlings was measured hourly during two 2-day watering cycles from before sunrise to after sunset. Seedlings were severed at the soil line and immediately tested by using a modified pressure chamber (5) (Soil Moisture Equipment Corp., Santa Barbara, CA).

To test for the presence of the pathogen in the surface soil, samples of sufficient size were collected to a depth of 6 cm at each 6-mm vertical interval to fill five 10-cm-diameter pots. Three sugar pine seeds, surface sterilized in 30% H_2O_2 for 1 hr, were planted in each of the pots which were then maintained in the greenhouse at 27 C. After 6 wk the number of seedlings that had died from hypocotyl rot was recorded.

Hyphal growth rate in soil. Estimated hyphal growth at 6-mm-depth intervals over a 2-day period was derived as follows. Mean hourly temperature and ψ_{soil} from field measurements (Figs. 1 and 2) were tabulated. Temperatures for each depth interval were taken as the average of the two measurements at the upper and lower boundary of each interval. Calculations of relative growth, summed for each hour of a 2-day irrigation cycle, were derived from a previous study (3) of the relationship of temperature and ψ to hyphal growth of the pathogen (4).

Effect of temperature and water potential on disease development. Surface soil was collected from a forest nursery that had been fumigated the previous fall and found to be free of the hypocotyl rot organism by periodic greenhouse bioassays (2). This soil was screened through a 1-mm sieve and used directly as noninfested soil.

Inoculum was prepared by first grinding barley straw to pass through a 1-mm screen. The ground straw (50 g) was then added to flasks and moistened with 2 ml of 0.025 M asparagine per gram of straw followed by autoclaving for 1 hr on each of two consecutive days. The straw was then inoculated with a spore suspension of the M-2 hypocotyl rot isolate (2), incubated for 3 wk under fluorescent illumination, dried, and again passed through a 1-mm screen. Infested soil was prepared by thoroughly mixing the ground straw inoculum with soil at the rate of 1 g/10 L of soil (~1:10,000, w/w).

A system was designed to allow temperature regulation of the surface 4-cm of soil while minimizing temperature effects at greater

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

©1985 The American Phytopathological Society

depths. Wooden flats 14 cm deep were filled with a sterilized mixture of perlite and U.C. mix (4) (1:2, v/v) to a depth of 9 cm. A layer of polyester batting 3 cm thick (1 cm thick under final soil weight) was placed over the soil mix to provide an insulation barrier between the soil levels. One centimeter of infested soil was then added above the polyester batting. Three heating cables (Chemelex 10 PTV; Chemelex, Redwood City, CA) were laid at 5-cm intervals on the soil which was then covered with an additional 3 cm of infested soil. The polyester layer did not appear to have an adverse effect on root growth.

Sugar pine seeds were collected from California seed zones, stratified, and surface sterilized as described above. The seeds were kept moist at room temperature and used for planting when germination commenced. Thirty seeds were planted in each flat between the heating cables 1.7 cm deep at 3.5-cm intervals.

A thermostat sensor was placed 1 cm from the seed row at planting. When 90% of the seedlings had emerged, 1-cm-thick polyester batting was placed on the soil surface and around the seedlings for heat and moisture retention. Thermocouples were buried in the bottom layer at four locations adjacent to the seedlings and just above the surface polyester in all flats to monitor temperatures throughout the system. Two similarly prepared flats, one with noninfested soil and the other without heating coils, were used as controls.

Soil was maintained at near field capacity in the upper level by burying one end of a heavy cotton wick in each corner of the flat and immersing the other end in a trough of distilled water beside each flat. The bottom soil level was kept moist by immersing the flats in a pan of shallow water just deep enough to contact the soil mix. Soil samples were removed periodically for determinations of water content and water potential as described above. Illumination (12 hr/day) was provided by fluorescent light (3.72 W/m^2) at a height of 0.5 m.

After 90% of the seedlings had emerged and the surface polyester was in place, heat was applied to the surface soil via the buried cables and controlled by a thermostat designed to avoid temperature surges. Temperatures of 29 or 34 ± 0.5 C were maintained in the heated surface soil, which corresponded to the optimum and that temperature above optimum at which radial growth in soil was reduced by 50% relative to the optimum, respectively. These temperatures were determined from a study in which measurements were made of hyphal growth through soil at near field capacity (3). Soil temperature in the unheated flat was 23 ± 0.5 C. Air temperature was maintained at 24 ± 1 C.

As seedlings died they were removed from the flats and inspected for hypocotyl rot symptoms. Five dead seedlings from each flat were surface sterilized and plated on PDA to confirm the causal agent. After 14 days, all seedlings were removed, inspected for hypocotyl lesions, and those with definite sunken lesions were recorded. The experiment was run a total of four times.

To determine if heating predisposed seedlings to infection, seedlings were grown in heated noninfested surface soil as described previously. After 90% of the seedlings had emerged, the surface soil was heated to 34 ± 0.5 C for 4 days and then cooled to 24 ± 0.5 C. The top 3 cm of soil surrounding the seedling hypocotyls were immediately removed and replaced with infested soil that had been kept moist at 24 C for 1 wk before use. An identical flat was set up and treated similarly, except that no heat was applied before the addition of the infested soil. After 14 days, all seedlings were removed, inspected for hypocotyl lesions, and those with definite sunken lesions were noted. The experiment was run a total of four times.

Water stress of the magnitude measured under field conditions was induced in seedlings to determine if this factor increased seedling losses to hypocotyl rot. Two seedling flats were prepared as for the heated-flat experiments. After 90% of the seedlings had emerged, one flat was covered with a polyethylene tent to increase humidity. Plastic film was placed over the surface polyester batting of the other flat to avoid excessive soil drying, and air at 33 C was blown across the seedlings during the 12-hr light period to induce water stress. The heated air was vented away to avoid heating the tent-covered flat. Air temperature inside the unheated plastic tent was 30 ± 1 C, and the surface soil temperatures in the heated and unheated flats varied an average of 1.5 C. At 2 and 7 days after the water stress was applied, five randomly selected seedlings from each flat were cut at the soil line midway through the hot air cycle, and ψ was measured by using the pressure chamber. After 14 days the number of dead seedlings or with definite lesions from each flat was determined. The experiment was run a total of four times.

RESULTS

The mean diurnal nursery soil temperature fluctuations from two 2-day irrigation cycles are illustrated in Fig. 1. The temperature range decreased rapidly with increasing soil depth, as would be expected. Surface temperatures ranged from 5 to 59 C, while at 30 mm the range was from 13 to 32 C. As with soil temperature, fluctuations in soil ψ decreased with increasing soil depth; they ranged from −0.5 to −150 bars at the surface and from −0.8 to −24 bars at the 24–30 mm depth (Fig. 2).

Plant ψ measured in the field ranged from −1.0 ± 0.2 bars before sunrise to −9.3 ± 0.7 bars at 1600 hours during the first afternoon; and −1.4 ± 0.4 bars before sunrise to −12.2 ± 0.5 bars at 1500 hours during the second day after watering.

Approximately 70% of the seedlings died from hypocotyl rot when grown in soil from all depths sampled. Although the sample size was not large enough to distinguish relative populations in all strata, it clearly indicated the presence of the pathogen at all levels.

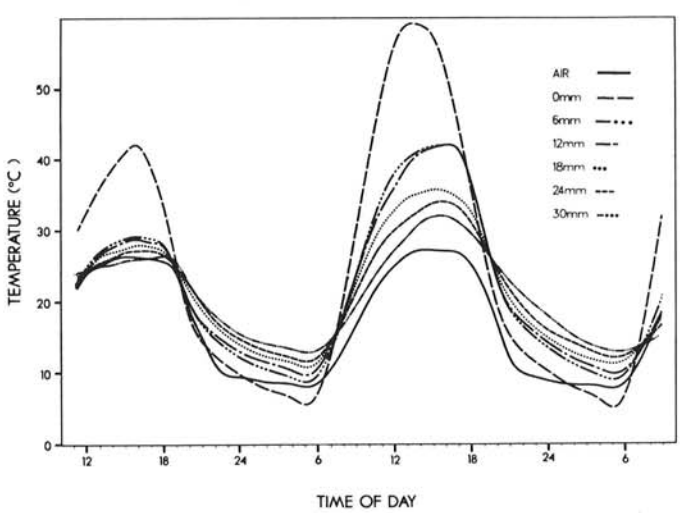

Fig. 1. Average temperatures recorded in air (1.3 m above ground level) and at six soil depths during two 48-hr periods at Magalia, CA.

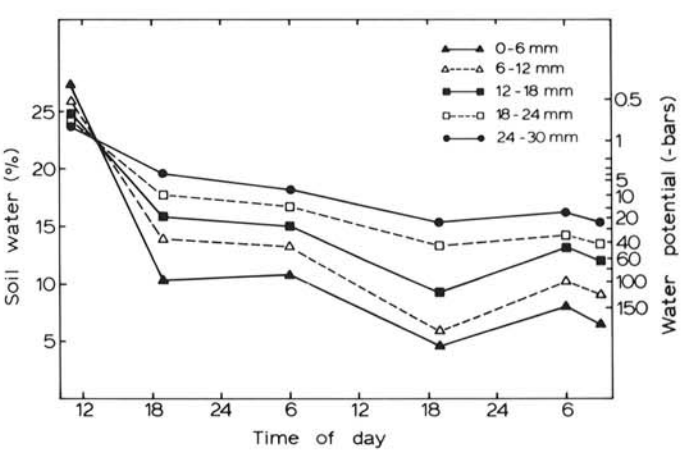

Fig. 2. Soil water status of a pine nursery soil at five depths following irrigation. Values are the means of samples taken during two 48-hr periods.

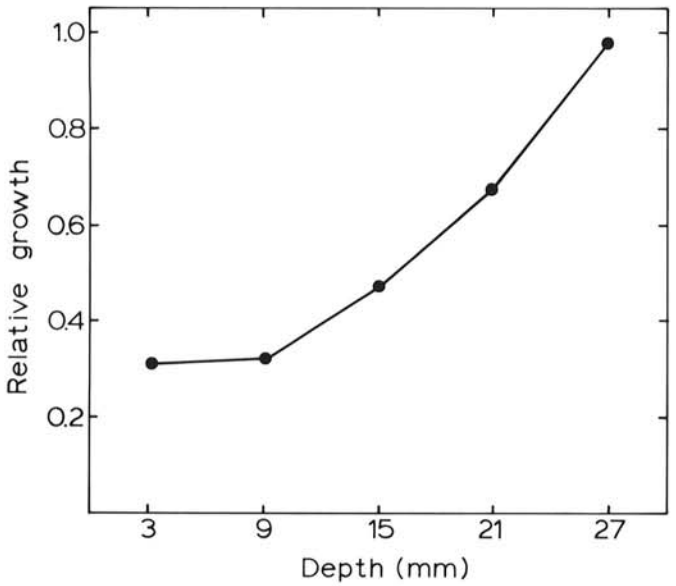

Fig. 3. Estimated relative linear growth of *Fusarium oxysporum* during a 48-hr watering cycle as a function of soil depth. See text for details of calculating relative growth.

Estimated hyphal growth rate in soil. Calculated estimates of cumulative 2-day growth rates at depths of 0–6, 6–12, 12–18, 18–24, and 24–30 mm are plotted for mean depths of 3, 9, 15, 21, and 27 mm in Fig. 3. Growth would be severely restricted above 10 mm and increase rapidly below that depth. At the average depth of lesion formation (17 mm), growth would be nearly twice (170%) the rate above 10 mm. Hyphal growth would continue to increase with increasing depth to the maximum calculated depth of 27 mm. In the first two depth intervals, down to 12 mm, ψ and temperature declined so rapidly that there would be no significant growth during the first night after irrigation. Below 12 mm, conditions remained favorable for growth throughout the first night, nearly doubling the time available for growth above the 12 mm depth, while at 25–31 mm, conditions remained favorable through the second night.

Influence of temperature and water potential on disease development. Forty one percent of the seedlings grown in heated flats that were maintained at 29 C (optimum for fungal growth) were diseased (dead or with obvious lesions) after 14 days. However, 76% of the seedlings grown at 34 C (temperature at which fungal growth was reduced by 50%) were diseased, an 85% increase over the proportion infected at 29 C. The unheated controls grown in infested soil had 5% disease, and the heated noninfested controls at 29 and 34 C had average losses of 4 and 7%, respectively.

Water potential of seedlings stressed by the hot air treatment averaged -11.2 ± 1.4 bars at mid-day, which is within 1 bar of field-grown plants; whereas the tent-covered controls averaged -6.7 ± 1.2 bars. There was no significant difference ($P = 0.1$) between the number of dead and lesioned seedlings after 10 days in the two treatments.

DISCUSSION

Because the hypocotyl rot pathogen was found at all depths in the surface soil, we determined the roles of environmental and host factors in regulating the depth at which lesions form. These conclusions are based on the assumption that the ability of the pathogen to infect and cause symptoms is related to its growth in soil. Water potential was found to be a major factor limiting fungal growth, becoming more restrictive in the shallowest stratum (0–12 mm) which dried most quickly. Here growth ceased within no more than 6 hr after each irrigation. Temperature, the other major environmental variable in the surface soil, also played an important role in affecting disease development. The fungus was able to grow rapidly in the warm wet soil during the first afternoon following irrigation. During the first night, temperatures declined more quickly at the surface; however, even at greater depths (up to 30 mm) growth would be greatly reduced for most of the night. During the second day, the top 6 mm reached very high temperatures and would be restrictive or lethal to the fungus; moreover, the soil had already become too dry for growth at any temperature. Only below 18 mm was the water potential high enough to allow growth during the second day. At this depth, temperature was higher than optimum for 5 hr at most, but never high enough to arrest fungal growth. Plant water deficits, of the magnitude recorded in the field, apparently were not a factor that affected host susceptibility.

Unlike water potential, temperature affected host susceptibility. Raising the temperature 5 C above the optimum for growth of the pathogen nearly doubled disease incidence, which suggests that heat predisposed the hypocotyl tissue to infection. This localized predisposition was of relatively short duration after the heating was terminated; however, a long-term effect is not required as the predisposing temperatures occur daily in the field.

In summary, the following factors influenced depth of lesion formation. Both temperature and moisture were more favorable for fungal growth at greater soil depths, which alone would increase the frequency of deeper lesions. However, lesion depth has a lower limit because root tissue, unlike hypocotyl tissue, is resistant to infection (2). On the other hand, host predisposition would tend to favor lesion formation closer to the surface. Thus, the characteristic depth of lesion formation is a product of counteractive environmental determinants in the top 5 cm of soil and is a function of both pathogen and host response to the environment.

That variations in air temperature, incident radiation, and frequency of irrigation would alter the relationships discussed here is recognized; however, these relationships are based on the conditions encountered when the disease was most severe. Results and conclusions from this study are useful in predicting the influence of an altered environment caused either by natural processes or management procedures, such as shading or changes in irrigation schedules, on disease development. These management techniques are now being practiced to reduce disease incidence.

LITERATURE CITED

1. Baver, L. D., Gardner, W. H., and Gardner, W. R. 1972. Soil Physics. John Wiley & Sons, Inc. New York. 498 pp.
2. Brownell, K. H., and Schneider, R. W. 1983. Fusarium hypocotyl rot in California forest nurseries. Plant Dis. 67:105-107.
3. Brownell, K. H., and Schneider, R. W. 1984. Roles of matric and osmotic components of water potential and their interaction with temperature in the growth of *Fusarium oxysporum* in synthetic media and soil. Phytopathology 75:53-57.
4. Matkin, O. A., and Chandler, P. A. 1957. The U.C.-type soil mixes. Pages 68-85 in: The U. C. System for Producing Healthy Container-grown Plants. K. F. Baker, ed. Calif. Agric. Exp. Stn. Ext. Serv. Man. 23. 232 pp.
5. Scholander, P. F., Hammel, H. T., Bradstreet, E. D., and Hemmingsen, E. A. 1965. Sap pressure in vascular plants. Science 148:339-346.