A Mist Generator and Environmental Monitoring System for Field Studies on Shothole Disease of Almond

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ABSTRACT

A mist generator controlled by a datalogger was constructed to study shothole disease of almond trees, caused by *Wilsonomyces carpopilus* (*Stigmina carpopilus*). The system wetted leaf surfaces on selected shoots and recorded temperature, leaf wetness, relative humidity, rain, wind speed, and wind direction. The mist generator consisted of a pressurized water source and reservoir, a delivery system, and a datalogger control system that monitored periods of leaf wetness and regulated mist regimes. Conditions conducive to infection of leaves of almond cultivars Nonpareil and Carmel by *W. carpopilus* were generated in the field by controlling duration of mist bursts, intervals between bursts, and length of time that leaves remained wet. Disease was most severe (3.1 lesions per leaf) when leaves were misted for 3 or 4 sec every 2.5 or 5.0 min for 16 hr overnight at temperatures of 10-30 C (average 14-17 C). Fewer lesions developed with longer bursts of mist (which removed conidia from leaves), longer intervals between mists (which allowed leaf surfaces to dry), or wet periods of less than 16 hr. Infection (averaging 3.5 lesions per leaf) also occurred when two different electrical conductance leaf wetness sensors were used to govern mist intervals during 16-hr wet periods.

A wide range of electronic sensors and output-recording dataloggers have been developed and used to monitor microclimate in studies of plant diseases (8,9,12-14). Many of these microcomputer-controlled systems in plant pathology were developed to predict disease occurrence based on models driven by environmental parameters such as temperature, leaf wetness, and relative humidity (8,9). These systems and sensors, however, have not been used to induce disease in the field by generating and monitoring environmental conditions needed for infection.

In California, spring rains favor infection of young leaves of almond (*Prunus dulcis* (Mill.) D. A. Webb by *Wilsonomyces carpopilus* (Lév.)) Adaskaveg, Ogawa, & Butler (syn. *Stigmina carpopilus* (Lév.) Ellis) (1). The disease, shothole, is initiated or intensified on flower hypanthia, fruit, leaves, or (rarely) twigs during wet periods (10,16). Irrigation systems with high-angle sprinklers that deposit water on leaves and fruit favor disease development (2).

Control of this disease could be enhanced if the environmental conditions conducive to infection were more precisely known. The objective of this study was to develop a mist generator controlled by an environmental monitoring system to produce and monitor wet periods conducive to infection of almond leaves by *W. carpopilus*.

MATERIALS AND METHODS
Plant material. Adjacent rows of Carmel and Nonpareil almond trees were inoculated with conidia of *W. carpopilus* and misted with the mist generator in 1987 and 1988. The trees were 6-7 yr old and 5-6 m tall. They were planted 7 m apart and were furrow-irrigated.

Equipment. The mist generator and control system (Fig. 1) consisted of a reservoir and pressurized water source, a delivery system with four independent main lines, and a control system/weather station consisting of a datalogger (Model 21X, Campbell Scientific Inc., Logan, UT) and weather sensors. The water source was a 210-L polyethylene storage tank filled with distilled water and fitted with a 12-V direct-current, duplex diaphragm pump (Model 2000-637, FlowJet Corp., Irvine, CA), a prepressurized, hydropneumatic tank (Well-X-Trol Model WX-250, Amtrol Inc., West Warwick, RI), a pressure gauge, and a 100-mesh filter (No. 124, Spray Systems Co., Wheaton, IL). These components were connected with 2-cm polyvinyl chloride pipe. The delivery system consisted of five 12-V direct-current solenoid valves (Hydro-Rain, Laguna Niguel, CA), one manual valve per reservoir tank, one 2-cm check valve, 1.3-cm polyethylene drip hose for main lines, polyethylene tubing 32 mm in diameter for risers, drip tubing connectors, citrus mister stakes (#IPS0409, Hardie Irrigation, Laguna Beach, CA), and mist nozzles (Monarch M-2, W. A. Westgate Co., Inc., Davis, CA).

The control system/weather station for monitoring environmental conditions consisted of an enclosed control circuit (Fig. 2) similar in design to a relay circuit by Campbell Scientific (4), a 12-V, 105-A-hr battery, a datalogger (Model 21X, Campbell Scientific), and the following sensors: four 8-m, 32-gauge Cu-Cn thermocouples (15), three Teflon-Ni-Cr leaf wetness sensors, one grid-type (Model 237) leaf wetness sensor (5,7), one ambient temperature and relative humidity probe (Model 207) in a Gill radiation shield (Model 41002-3), one R. M. Young wind monitor (Model 05103) for wind speed and direction, and one rain gauge (Model RG2501). All sensors, except the Teflon-Ni-Cr leaf wetness sensors, were obtained from Campbell Scientific; the Teflon-Ni-Cr sensors were constructed similar to a previously developed sensor (12), using Teflon cylinders 50 mm long by 3 mm in diameter with two pairs of Ni-Cr wires attached along each cylinder so that individual wires alternated from either side of the cylinder and were equally spaced. Multiplier and offset factors for leaf wetness sensors were calculated based on wet and dry sensor readings and were incorporated into the datalogger program to convert the measured alternating-current conductance to a reading between 0 (when the sensor was dry) and 10 (when fully coated with water).

Thermocouple and leaf wetness probes were secured to tree branches. Shoots and sensors were misted with distilled water, and sensor readings were observed as the foliage dried. The sensor reading when all visible water had evaporated from leaves was used as a critical value in the datalogger program for the initiation of misting. The datalogger and other weather sensors were mounted on a 2-m tripod with a cross member. The datalogger was programmed to read sen-
sors (temperature, relative humidity, leaf wetness, and rainfall) every 60 sec and to record hourly and daily averages, highs, lows, and accumulations.

**Design of the mist generator.** The mist generator was designed to fit on a 1.3-m³ wooden pallet and was moved to orchard study sites in a pickup truck. Distilled water was transferred to the system from a 210-L polyethylene container on a truck. Water flowed from a valve at the base of the storage reservoir through a hose (2 cm in diameter) to the pump and into the hydropneumatic tank (Fig. 1). Pressure was maintained at 206–345 kPa.

In all experiments, four mist treatments (each with three replications) were implemented simultaneously with six nozzles (two per replicate) connected to each of the four main lines. The control box housed manual and automatic on-off switches for the system, fuses, and circuits for controlling the solenoid valves (Fig. 2). Leaves received mist from two nozzles positioned about 30–40 cm from a selected shoot in one of two ways. Shoots near the ground were tied to steel stakes (1 cm in diameter) and the mist nozzles, attached to additional stakes, were placed on either side of the shoot. Shoots higher in the canopy were placed in wire cylinders (40 cm in diameter by 60 cm long) made from poultry netting. A bamboo stick (1 cm in diameter) was attached across both ends of the cylinders with baling wire. Tree shoots placed through the cylinders were attached to the center of each bamboo stick to secure the shoot in the center of the cylinder. A nozzle (in a citrus mister stake) was mounted on either side of the wire cylinder and directed toward the shoot.

**Inoculum production and inoculation of almond cultivars.** A single-spore isolate of *W. carpophilus* (JEA637; Modesto, CA), obtained from diseased almond leaves, was used in all experiments. Conidia were produced in cultures grown on 4% potato-dextrose agar (PDA) (Difco) for 6 days in the dark and then placed 30 cm under 20-W fluorescent lights for 4–6 days. Conidia were collected by washing the cultures with autoclaved, glass-distilled water. Conidial suspensions were filtered, concentrated by centrifugation, and stored at −15 C.

Before inoculation of almond leaves, suspensions were thawed, diluted to 1 × 10⁶ conidia per milliliter, and held in an ice bath at 0–1 C (11). Inoculations were performed within 1 hr after inoculum preparation. In all studies, we inoculated the undersides of mature leaves by spraying them with an airbrush (Model H, Paasche, Chicago, IL) using carbon dioxide at a pressure of 105 kPa as the propellant. This method deposited about 300–400 conidia per square centimeter of leaf surface in an even distribution of small droplets. The percentage germination of conidia was evaluated on water agar, and after inoculation and wetting, the surfaces of selected leaves were examined for the presence or absence of conidia by epifluorescence, bright-field microscopy.

**Moisture treatments.** The datalogger was programmed to control wet periods by varying the duration of and interval between bursts of mist. Treatments were designed to determine the influence of length of wet period, duration of mist burst, and interval between bursts on leaf infection. The treatments were as follows: wet periods of 0, 6, 8, 10, 12, 14, or 16 hr with 4-sec mist bursts at 5-min intervals; mist bursts of 2, 3, 4, 5, 7, 10, or 15 sec at 5-min intervals for 16 hr; intervals of 2.5, 5, 10, 20, 30, or 60 min between 4-sec mist bursts for 16 hr; and 20-sec mist bursts at 10-min intervals for 16 hr. Foliage of the zero treatment was inoculated but not misted.

Treatments were initiated in late afternoon and concluded the following morning. In all experiments, treatments were performed on consecutive days and were replicated on three trees. The experiments were conducted in 1987 on Carmel and Nonpareil trees and were repeated twice in 1988 on Carmel trees. Data were analyzed by linear regression.

The datalogger was also programmed to govern mist regimens based on readings from leaf wetness sensors on shoots. Mist bursts of 4 sec triggered by critical values of 3.0 and 4.0 were used with the Teflon-Ni-Cr and grid leaf wetness sensors, respectively. For a period of 16 or 72 hr, whenever sensor readings fell below the critical values (minimum interval 2.5 min), the solenoid valve controlling water flow was signaled to open for 4 sec. The experiment was repeated once in 1987 and twice in 1988 on Carmel trees. Data were analyzed by analysis of variance. Mean separations were performed using Duncan's new multiple range test.

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**Fig. 1.** Schematic drawing of mist generator, showing the 210-L reservoir (RES), 2-cm reinforced hose (H), 12-V direct-current pump (PUMP), 2-cm polyvinyl chloride tubing (T), hydropneumatic pressure tank (HPT), pressure gauge (G), check valve (CK), filter (FIL), solenoid control valves (SV1–SV4), one-way valves (V), solenoid release valve (SRV), 1.3-cm polyethylene drip lines (L1–L4), 0.3-cm polyethylene hose (risers) (R), spray nozzles (SN), 12-V battery (BAT), and relay control box (RCB).

**Fig. 2.** Schematic diagram of control circuits for battery, pump, and one solenoid valve. Standard symbols are used for electronic components. Switch 1 (S1) is for automatic control, switch 2 (S2) is for manual control, and switch 3 (S3) controls the pump.
Disease evaluation. In all experiments, disease was assessed 10–14 days after a treatment, following the method of Shaw et al (11). Lesions were counted on 10 representative leaves from each treated shoot. In all experiments, a random sample of 30 lesions was surface-sterilized (1 min in chlorine at 400 µg/ml) and plated on acidified PDA (0.075% lactic acid) for identification of the pathogen.

RESULTS

Function of the mist generator. The datalogger controlled the mist generator system by sending a 5-V signal to the control box at programmed time intervals or when leaf wetness sensor readings indicated dry foliage. The control box completed the 12-V circuit to open the appropriate solenoid valve for one of the main lines or the pressure release line. When a control valve was opened, water flowed through a main line and risers to the mist nozzles. After the control valve was closed, the release valve was opened for 3 sec to reduce pressure in the main lines and risers, which prevented dripping from the nozzles. Water from the release valve circulated back into the reservoir. A mist regime with 12 nozzles delivering 4-sec bursts every 2.5 min for 16 hr required about 40 L of distilled water.

A program for the datalogger to control timed mist intervals is shown in Figure 3. When leaf wetness sensors controlled mist timing, another program was used (Fig. 4). Both programs ran for a specified number of hours, which varied by experiment.

Mist treatments and induction of shoothole disease. Temperatures during the experiments averaged 20 C (range 10–27 C), and relative humidity averaged 30% (range 15–45%). No rain was recorded during the experiments.

A 16-hr wet period (3- or 4-sec bursts of mist at 5-min intervals) resulted in the most severe disease (Fig. 5). Lesions developed after all wet period treatments except the control and ranged up to 3.1 lesions per leaf in a 16-hr treatment in both seasons. Mist bursts of 3 and 4 sec (at 5-min intervals for a 16-hr wet period) resulted in the most lesions, averaging 2.7 and 2.3 lesions per leaf, respectively (Fig. 6). Frequency of infection declined with progressively longer mist bursts up to 15 sec, and no disease occurred with 20-sec bursts. Intervals of 2.5 and 5 min between mist bursts (with 4-sec bursts for 16 hr) resulted in averages of 2.1 and 2.3 lesions per leaf, respectively (Fig. 7). Intervals of 10 min or longer resulted in progressively fewer lesions per leaf.

Disease incidence with mist regimes governed by the grid or Teflon-Ni-Cr sensors (with 4-sec bursts and a minimum interval of 2.5 min) was not significantly different (P = 0.05). These regimes resulted in a range of 2.0–6.9 (1987) and 1.3–3.5 (1988) lesions per leaf for both 16- and 72-hr treatments. Differences between 16- and 72-hr wet periods were not significant when mist regimes were governed by leaf wetness sensors. No significant difference in disease incidence was observed between cultivars Nonpareil and Carmel.
Shothole symptoms were absent in inoculated-dry and noninoculated-mistted treatments. Disease was absent or incidence very low when mist bursts lasted longer than 5 sec; these treatments resulted in water runoff and removal of inoculum. Conidia were observed on inoculated leaves in all treatments with mist bursts of 5 sec. No conidia were found on noninoculated leaves or inoculated leaves that received mist bursts of 7, 10, or 15 sec every 5 min or 20 sec every 10 min for 16 hr. In all studies, germination of conidia exceeded 80% after 24 hr, and *W. carphophilus* was isolated from 70–80% of the lesions that were sampled. Other fungi isolated included species of *Alternaria*, *Cladosporium*, and *Penicillium*.

**DISCUSSION**

The mist system produced and monitored environmental conditions that allowed infection by *W. carphophilus* and the development of shothole on leaves of almond. Drip irrigation fittings used for connecting main lines, risers, and nozzles of the mist generator readily allowed modifications of the delivery system for mist treatments on selected trees. The delivery system was designed with five solenoids controlling four main lines and one release line. Because the data logger (Campbell 21X) has six output ports for controlling external electrical devices, a maximum of six solenoids controlling any combination of main and release lines could be adapted to the system; however, a larger reservoir, resizing of the hydropneumatic tank, and program modifications for the data logger would be required.

Wire cylinders provided specific placement and adjustment of spray nozzles around selected shoots. The cylinders were light and easily moved from shoot to shoot and offered little wind resistance, thus minimizing potential tree injury and changes in microclimate.

The system wet plant surfaces by misting shoots from two sides. Three parameters of water application were critical for determining optimal condi-

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**Fig. 4.** Portion of a supplemental datalogger program for controlling solenoid valves based on signals from a leaf wetness sensor of the mist system. A portion of supplemental user programs (*2* and *3*) and entry codes for the Campbell 21X datalogger are shown. User program 1 (*1*) for weather monitoring is not shown. The interval between mist bursts is based on readings of a leaf wetness sensor, with a selected critical value of 3.0. Each of the four lines can be controlled by four different leaf wetness sensors. The minimum interval between mist bursts is 4.0 min (240 sec). Length of mist bursts (set for 3 sec) is based on the datalogger counter. Wet period is controlled by the number of hours into the year (steps 11 and 12). Subroutines in *3* turn the pressure release line on and off. Exec = program execution interval, Count = execution interval counter, Loc = memory location, Mod = modify by, F Value (F Val) = comparison function, Sec = seconds (based on counter), t = time.

**Fig. 5.** Regression of the natural logarithm of the average number of lesions per 30 leaves on the duration of the wet period after inoculation of Carmel almond leaves with *Wilsonomyces carphophilus* in one experiment (*P* = 0.05). Mist was applied in 4-sec bursts at 5-min intervals. Disease was evaluated 14 days after inoculation. Other experiments gave similar results.

**Fig. 6.** Regression of the natural logarithm of the average number of lesions per 30 leaves on the length of mist bursts during infection of Carmel almond leaves by *Wilsonomyces carphophilus* in one experiment (*P* = 0.05). Mist was applied at 5-min intervals for 16 hr. Disease was evaluated 14 days after inoculation. Other experiments gave similar results.

**Fig. 7.** Regression of the natural logarithm of the average number of lesions per 30 leaves on the interval between mist bursts during infection of Carmel almond leaves by *Wilsonomyces carphophilus* in one experiment (*P* = 0.05). Mist was applied in 4-sec bursts at varying intervals during a 16-hr wet period. Disease was evaluated 14 days after inoculation. Other experiments gave similar results.
tions for shothole development: duration of mist bursts, interval between bursts, and the amount of mist foliage was wet. Mist bursts of 3-4 sec at 2.5- to 5-min intervals for 16 hr were optimal. Shorter intervals might be needed during the day or on windy nights; use of the mist system under such conditions would warrant either periodic changes in the programmed interval between mist bursts or the use of a leaf wetness sensor to activate the valves.

Less disease developed than we expected, given that 300-400 conidia per square centimeter were applied to leaves. Shaw et al (11), using the same inoculation technique, obtained an average of 50-60 lesions per leaf on inoculated plants that were held in a dew chamber with leaves constantly wet for 16 hr and then incubated in growth chambers at 15 C. In field studies using a static bagging technique, Shaw et al (11) obtained an average of 15.3 lesions per leaf at average temperatures of 16.4 C. In our study, cyclic applications of mist may have affected the distribution and number of conidia on leaves. Lower disease incidence may have been the result of clustering of conidia from surface tension of receding water spots on drying leaves or removal of conidia by water runoff (however, water runoff was reduced with short mist bursts). Conidia germinated readily under laboratory conditions, using the distilled water used for the mist system. When conditions of wetness are modified, other environmental factors, such as ultraviolet radiation and wind, may affect spore survival.

Sensor placement and the selection of critical values used in the datalogger program determine performance of the mist system when sensors are used to control water application. The critical values can be defined to generate wet conditions suitable for studies of other foliar diseases.

A variety of sensors can be used to monitor leaf wetness and govern the mist regimes. We observed that water evaporated from the leaf wetness sensors (Teflon-Ni-Cr and grid types) and from almond leaves at similar rates. Other researchers have suggested that coating the sensors with latex paint may aid in obtaining sensor readings that are more representative of leaf wetness conditions (7,13,15).

The programmable mist system described herein is a versatile tool for field experiments on diseases that require specific conditions of wetness and temperature for infection and disease development. In our experiments, 14-16 hr of wetness at temperatures between 8 and 25 C were required for infection; this result agrees with the findings of Shaw et al (11). Infections of sour cherry by Blumeriella jaapii (Rehm) von Arx (6), of apple by Venturia inaequalis (Cke.) Wint. (9), and of stone fruit by Monilinia spp. (3) have also been shown to be influenced by temperature and wetness. In the spring of 1987, a preliminary experiment with our mist system resulted in significant infection of blossoms of apricot (P. armeniaca L.) by Monilinia laxa (Aderhold & Ruhland) Honey after inoculation and a 16-hr wet period (unpublished). The mist generator and environmental monitoring system may be valuable in the study of these and other diseases, especially in dry years or in semiarid climates with infrequent or little rainfall during the growing season. The use of this system may also facilitate studies on the efficacy of fungicides.

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LITERATURE CITED