# Influence of Wetness Period and Temperature on Infection and Development of Shot-Hole Disease of Almond Caused by *Wilsonomyces carpophilus*

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## ABSTRACT

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Temperature and duration of wetness period during the inoculation period influenced the development of shot-hole disease on almond leaves caused by *Wilsonomyces carpophilus* (*Stigmina carpophila*). In controlled environment studies, a 14-hr wetness period resulted in 0.1 and 45.0 lesions per leaf after 10 days at 8 and 22 C, respectively. Extended wetness periods during the infection period increased the number of lesions per leaf regardless of the temperature. Numbers of lesions increased as temperature was increased from 8 to 22 C for wetness periods greater than 12 hr. At 8 C, 4.7 and 45.6 lesions per leaf developed after wetness periods of 30 and 48 hr, respectively, and, at 15 C, 110.0 lesions developed at 15 C after a wetness period of 28 hr. Temperature after the infection period influenced symptom expression, the rate of lesion development, and lesion abscission, but not the number of lesions formed. Lesion abscission, or shot-hole, was significantly higher at 22 C (72.0% of lesions showing abscission) than at 8 C (0.3%) and 15 C (3.9%). Additional wetness period after an initial infection period resulted in a greater number of lesions and a higher disease index after 8 days at 8, 15, and 22 C and the formation of sporodochia after 16 days at 22 C. Field studies under ambient day and night temperatures and wetness periods of 10–12 hr and 14–16 hr resulted in an average of 6.6 and 16.0 lesions per leaf, respectively. In field studies, additional wetness periods also resulted in an increase in leaf infections and higher disease index. Germination percentages of conidia of *W. carpophilus* averaged 85.3% in 4 hr and 99.2% in 8 hr at temperatures between 10 and 30 C. Isolates tested had similar pathogenic reactions on almond leaves of cultivars Ne Plus Ultra (chamber tests), Carmel, Mission, and Nonpareil (chamber and field tests). A preliminary predictive model for disease was developed based on temperature and wetness period duration when inoculum was present.

*Additional keywords: Coryneum beijerinckii*, epidemiology, disease modeling, *Stigmina carpophila*.

Shot-hole disease, caused by *Wilsonomyces carpophilus* (Lév.) Adaskaveg, Ogawa, & Butler (1) (synonyms *Stigmina carpophila* (Lév.) M. B. Ellis and *Coryneum beijerinckii* Oud.) is a serious disease of *Prunus* species in many of the temperate to semiarid regions of the world (3,5,13,17,19,23,26). The fungus infects leaves and fruit and to a lesser extent twigs, the calyx of blossoms, and possibly buds of almond (*Prunus dulcis* (Mill.) D. A. Webb) under favorable conditions of wetness and temperature (9,23). During the spring, rainfall provides conditions necessary for primary infection of developing leaves and blossoms. In California, the disease has been shown to cause significant yield losses when trees develop leaf and fruit infections (10). Infections of young leaves or petioles can cause leaf drop while severe infections can cause terminal shoot blight (18,24). Secondary infections may occur throughout the growing season as a result of additional rainfall or sprinkler irrigations (4,23). However, fruit infections during the summer do not result in fruit drop or in a reduction in the size or weight of the endosperm or kernel (10). During fall rains in California, *W. carpophilus* infects and forms lesions on leaves of almond. Conidia from sporodochia of these lesions can overwinter on the tree in healthy buds and function as primary inoculum for spring infections (9).

One to three fungicide applications are made on 172,000 ha at an annual expense of $5–15 million for control of shot-hole disease of almond in California (25). If the effects of temperature and wetness on disease development on various host tissue and cultivars could be more clearly defined, it may be possible to develop a disease forecasting system that could determine timing of fungicide sprays, reduce unnecessary applications, and optimize chemical control practices (15). The objectives of this study were to determine the influence of temperature and wetness period on the germination of conidia and on the development of shot-hole of almond leaves; and to use this information to develop a preliminary model to predict intensity of disease.

## MATERIALS AND METHODS

**Isolates, inoculum preparation, and cultural studies.** Isolates of *W. carpophilus* were collected from stone fruit crops in central California. Isolates used were: JEA637, collected from leaves of almond, Modesto, Stanislaus County; DAS871, DAS872, and DA5873 collected from peach twigs (*P. persica* (L.) Batsch), apricot fruit (*P. armeniaca* L.) and leaves of almond, Davis, Yolo County, respectively. Pure cultures were tested for pathogenicity by inoculation of almond leaves and reisolation from developing lesions for comparison to parent isolates. Isolates were grown on potato-dextrose agar (PDA) (Difco Laboratories, Detroit, MI) and stored at 3 C.

Conidia were produced by growing cultures of *W. carpophilus* on PDA for 6 days in the dark and for 4–6 days 30 cm below 20W fluorescent lights. Conidia were collected by washing the cultures with sterile distilled water. Conidial suspensions were filtered through cheesecloth, centrifuged, and frozen in sterile distilled water at −15 C. Germination of conidia for each isolate was measured by pipetting 150 μl of a suspension containing $1 \times 10^5$ conidia/ml onto PDA. The plates were incubated at 0–1, 5, 10, 15, 21, 25, 30, and 33 C and examined after of 4, 8, and 24 hr. One hundred conidia were counted for each of three replications per isolate. The experiment was repeated once. A conidium was considered germinated when a germ tube was

at least 1.5 × the width of the conidium.

Germination of conidia and germ tube elongation were observed after wetting and drying treatments. Conidia were placed on cellophane (Type VII, E.I. du Pont de Nemours & Co., Wilmington, DE) attached to glass slides with paper clips, hydrated for 4 hr at 22 C by placing a filter paper wick under the cellophane in sterile distilled water in a moist chamber with >95% relative humidity (RH), and air dried for 20 hr at 22 C and 25% RH. Drawings of 20 conidia per experiment were made using a camera lucida on a Zeiss microscope before and after each hydration period for three cycles. This procedure was continued for five cycles and was repeated once.

**Inoculation of cultivars and disease evaluation.** Bare-root Carmel, Mission, Ne Plus Ultra, and Nonpareil almond trees on 5-37 rootstock were obtained from Fowler Nurseries, Inc., Lincoln, CA. Trees were pruned to a height of 30 cm and planted as needed in 4-L plastic pots. The trees were placed in a lathe house with overhead sprinkler irrigation until they reached a height of approximately 60 cm. Each tree developed four to eight actively growing shoots. Undersides of mature leaves were inoculated with a conidial suspension so that approximately 300–400 conidia were deposited per centimeter square of leaf tissue. For this, inoculum ($1 \times 10^5$ conidia/ml) was prepared from frozen conidial suspensions and was applied to leaves using an airbrush (Model H, Paasche, Chicago, IL) with carbon dioxide gas at a pressure of 9.8 PA as the propellant. Percent germination of conidia on water agar was evaluated after each inoculation treatment. All four almond cultivars were inoculated in growth chambers with either of the following isolates: JEA637, DAS871, and DAS872. Inoculated trees were placed in a dew chamber (Model I-60DL, Percival Manufacturing Co., Boone, IA) for 16 hr at 15 C, allowed to air dry for 15 min, and then transferred to a plant growth chamber at 15 C. Ten days after inoculation, eight leaves on each of two shoots were evaluated for the number of lesions per leaf. The experiment was repeated once.

Disease was quantified for each inoculated leaf by counting the number of lesions and assigning a disease index value representing the percentage of the leaf area infected. A disease index was developed based on the evaluation of 25 leaves by using digital image analysis on a Quantumet 900 (Lathaom Ltd., England). Leaves of different sizes, with different sizes and numbers of lesions were selected, and total leaf and necrotic areas were measured. Leaves were rated on a scale of 0–5 based on the percentage of diseased leaf area to the nearest 1% as follows: 0 = no infection; 1 = ≤1%; 2 = 2–5%; 3 = 6–10%; 4 = 11–20%; and 5 = >20% of total leaf area diseased. Color photographs of the analyzed leaves were then used as a standard curve to evaluate leaves. In all experiments, a random sample of 30 lesions was surface sterilized (1 min—400 μg/ml NaOCl) and plated on PDA amended with 0.075% lactic acid for isolation of the pathogen.

**Growth chamber studies with continuous wetness periods.** Potted trees of cultivars Ne Plus Ultra and Carmel were inoculated with isolate JEA637 and placed in a dew chamber for wetness period treatments at specific temperatures and durations. Treatments were arranged in a composite response surface design (6). After initial infection treatments, trees were air-dried approximately 15 min and transferred to growth chambers set at various temperatures. The specific treatments were (temperature [C] of infection period/duration of wetness during the infection period [hr]/temperature [C] during the incubation period for disease expression): 5/20/15, 8/14/8, 8/26/8, 8/14/22, 8/26/22, 8/26/15, 8/30/15, 8/40/15, 8/48/15, 15/20/5, 15/12/15, 15/20/15, 15/28/15, 15/20/25, 22/14/8, 22/26/8, 22/14/22, 22/26/22, and 25/20/15. Temperature and wetness periods were monitored in the dew chamber using a datalogger and four thermistors (Model 201, Campbell Scientific Inc., Logan, UT) and two teflon-nickel leaf wetness sensors (2). Hygrothermographs (Weathermeasure Inc., Sacramento, CA) in each plant growth chamber recorded temperature and relative humidity. Relative humidity ranged from 70 to 85% at night and 45 to 60% during the day. Incandescent and fluorescent lights were timed to provide a 12-hr photoperiod each day. Light levels were monitored periodically using a pyranometer (Model 50, Eppley Lab. Inc., Newport, RI) and a digital voltmeter (Model 163, Keithley Instruments Inc., Cleveland, OH). Light intensities of approximately 86.1 W $m^{-2}$ were maintained in each chamber. Ten days after inoculation, five leaves on each of three replicated shoots were evaluated for the number of lesions per leaf and were also given a disease index rating as described previously. The experiment was done once with Carmel and once with Ne Plus Ultra except for treatments 8/26/15, 8/30/15, 8/40/15 where Ne Plus Ultra was used for both experiments. Data were analyzed using Regression (REG), and Response Surface Regression (RSREG) procedures of the Statistical Analysis Systems (SAS) software (SAS Institute Inc., Cary, NC). A response surface was determined using spline fit and grid procedures of SAS.

**Growth chamber studies with interrupted wetness periods.** Thirty mature leaves on each of three replicated potted trees of cultivars Nonpareil and Ne Plus Ultra were inoculated with isolate JEA637 and the trees were placed in the dew chamber for a 16-hr wetness period at 15 C. After drying, trees were moved to growth chambers set at 8, 15, and 22 C. Additional wetness period treatments were performed by spraying selected trees with distilled water using a hand-held spray bottle and covering each tree with a large plastic bag. The plastic bag was sealed and leaves kept wet for 12 hr while the trees were in the dark. In each growth chamber, three trees remained dry with no wetness period treatment ("0"); three trees received two wetness period treatments ("2") in 8 days (treated every 4 days); and three trees received four wetness period treatments ("4") in 8 days (treated every 2 days). Data were collected by counting the number of lesions on each leaf daily for 5 days after inoculation. On day 8, leaves were given a disease index rating as previously described. The number of lesions with sporodochia were counted 14 days after inoculation. Totally and partially abscised lesions were counted and the leaves were photographed after 16 days to record differences in symptom expression. The experiment was done once with Nonpareil and once with Ne Plus Ultra. Lesion data were transformed with the square root transformation before analysis, while sporodochia and abscission data were analyzed as a percentage of the number of lesions per leaf. Data were analyzed using the GLM procedure of SAS.

**Field studies with continuous wetness periods.** Actively growing shoots of three almond trees (one shoot per tree) of each cultivar (Carmel, Mission, and Nonpareil) were inoculated with each of the three isolates of *W. carpophilus* used in the chamber studies. Trees were 6 yr old and were planted 7 m apart, in adjacent, furrow-irrigated rows, at the U.C. Davis Armstrong Experimental Field Station. Shoots were inoculated using the airbrush technique as previously described. After inoculation, a 10-mil plastic bag with the inside sprayed with distilled water was inserted in a brown paper bag and both bags were placed over the shoot for 16 hr. A wet paper towel was wrapped around the stem to provide wetness, and a wire tie secured both bags and paper towel to the shoot. For timed wetness period treatments, three replications (one shoot/tree/cultivar) were inoculated with isolate JEA637 and given wetness periods of 6, 8, 10, 12, 14, and 16 hr. Thirty-two gauge, Cu-Cn thermocouple temperature probes connected to a Campbell Scientific Model 21X datalogger were placed on treated shoots to monitor temperature during the experiments.

**Field studies with interrupted wetness period.** Alternating wet and dry treatments were also performed on trees in the field. Cultivars Carmel and Nonpareil were inoculated and placed in plastic/paper bags for 16 hr as previously described. Wetness period treatments corresponded to the 0, 2, and 4 treatments described in the growth chamber studies. Treatments were replicated three times (one shoot per tree per cultivar) and the experiment was repeated once. After 10 days, 10 representative leaves from each treated shoot were evaluated for disease severity as in the growth chamber studies. Data were analyzed using analysis of variance (ANOVA) and general linear models (GLM) procedures of SAS.

## RESULTS

**Conidial germination, pathogenicity of isolates, and susceptibility of cultivars.** Germination percentages for conidia of the four isolates of *W. carpophilus* were similar. At 10–30 C, more than 50% of the conidia germinated after 4 hr and 99.2% after 8 hr (Fig. 1). At 1, 5, and 33 C, germination was less than 5% after 8 hr. Percent germination of conidia after each inoculation in chamber and field studies was more than 90% after 24 hr on water agar medium at 20 C.

In growth chamber studies, no significant difference ($P > 0.05$) was observed in susceptibility between cultivars Carmel (84.6 average number of lesions per leaf), Mission (67.6), Ne Plus Ultra (77.0), and Nonpareil (66.3). Isolates JEA637 (81.0 average lesions per leaf), DAS871 (68.5), and DAS872 (70.5) showed similar pathogenicity and were not significantly different ($P > 0.05$) on all four cultivars studied. In field studies, no significant differences ($P > 0.05$) in susceptibility were observed among the cultivars Carmel (28.3 average lesions per leaf), Mission (15.3), and Nonpareil (23.0). Isolates JEA637 (31.1 average lesions per leaf) and DAS871 (29.5) were not significantly different; however, isolate DAS872 (6.0) was significantly different ($P \leq 0.05$) in pathogenicity on the three cultivars studied.

**Influence of temperature and wetness period during inoculation.** The number of lesions per leaf resulting from duration of wetness period and levels of temperature during the wetness period are shown in the three-dimensional scatterplot in Figure 2. Grouped

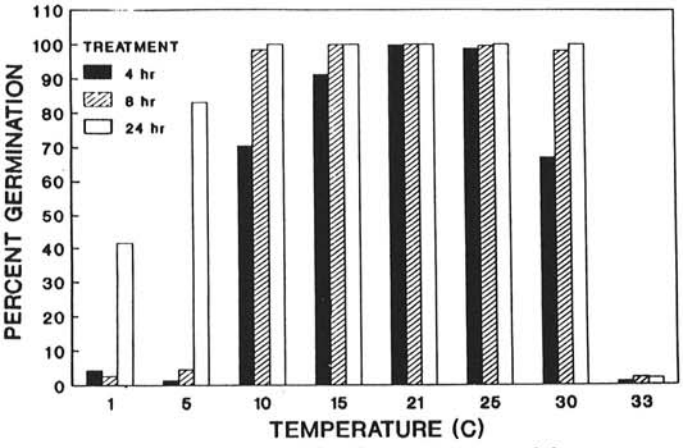


**Fig. 1.** Germination of conidia of *Wilsonomyces carpophilus* on potato-dextrose agar after 4, 8, and 24 hr of incubation at 1–33 C. Values are the average of four isolates: DAS871, DAS872, DAS873, and JEA637.

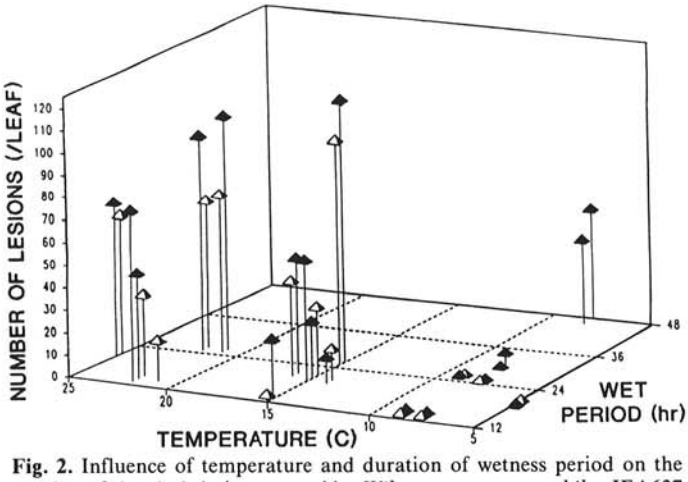


**Fig. 2.** Influence of temperature and duration of wetness period on the number of shot-hole lesions caused by *Wilsonomyces carpophilus* JEA637 on leaves of cultivars Carmel (open pyramids) and Ne Plus Ultra (solid pyramids). Trees were inoculated with conidia and incubated in a growth chamber. Grouped points are the average of three replications for each treatment (excluding post-wetness period temperature).

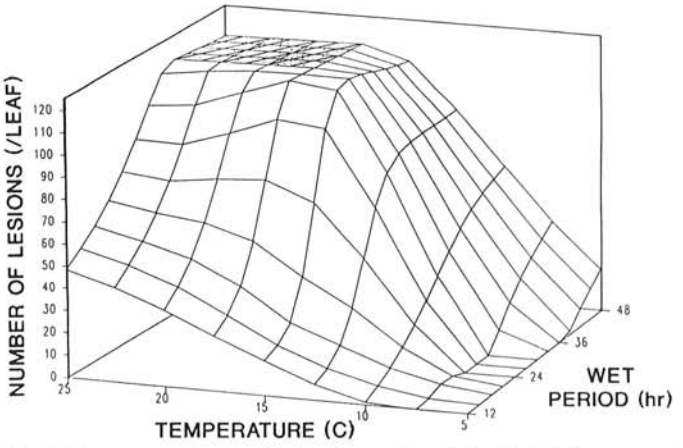


**Fig. 3.** Response surface predicting the number of shot-hole lesions caused by *Wilsonomyces carpophilus* JEA637 on leaves of cultivars Carmel and Ne Plus Ultra. Trees inoculated with conidia and given wetness treatments from 12–48 hr at temperatures of 5–25 C in dew and growth chambers. Shaded region represents extrapolated data.

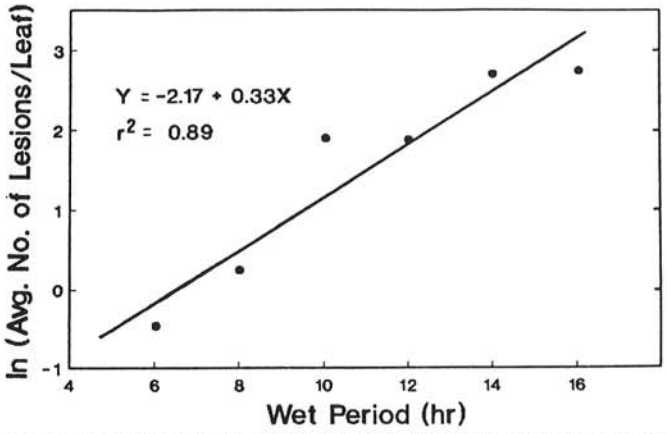


**Fig. 4.** Regression of ln of the number of shot-hole lesions on duration of the wetness period (hr) during infection of almond leaves by *Wilsonomyces carpophilus* JEA637 ($P \leq 0.05$). Disease incidence was measured as the number of lesions per leaf and evaluated 14 days after inoculation. $Y$ = ln of the number of lesions and $X$ = wetness period (hr).

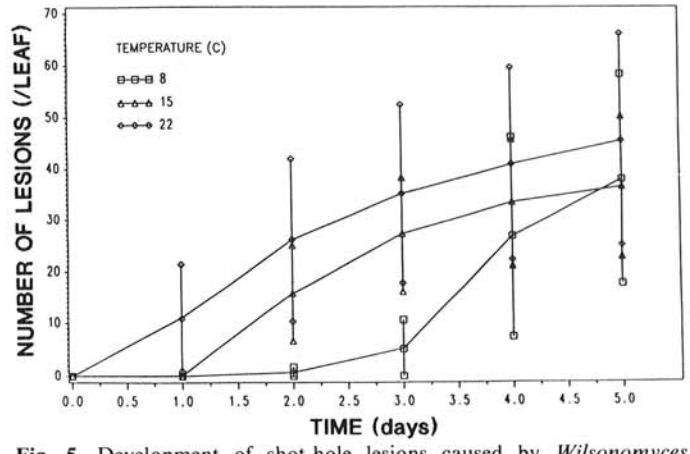


**Fig. 5.** Development of shot-hole lesions caused by *Wilsonomyces carpophilus* JEA637 on leaves of cultivars Ne Plus Ultra and Nonpareil after 5 days at temperatures of 8, 15, and 22 C. Trees inoculated with conidia, given a 16-hr wetness treatment at 15 C, and incubated in a growth chamber. Values represent average for all postinoculation wetness treatments.

points are the average of the replicates of each infection period treatment (temperature/wetness period) excluding post-temperature levels. Infection of almond leaves increased with increasing periods of wetness and with increasing temperature during the wetness period. The equation:

$$Y = (0.73T - 0.02T^2 - 0.19W + 0.004W^2 + 0.018TW - 4.05)$$

where $Y$ = number of lesions per leaf, $T$ = temperature (C) during the wetness period, and $W$ = length of wetness period (hr), described infection of leaves with a coefficient of determination ($R^2$) of 0.84. All estimated parameters of the equation were significant ($P \leq 0.05$). At ≤8 C and wetness periods <48 hr, there was an average of 1.5 infections for Ne Plus Ultra and 0.4 infections for Carmel; at 8 C with a 48-hr wetness period, an average of 45.6 lesions per leaf were observed. At 15 C, a similar number of lesions per leaf were observed with a 20-hr wetness period. More than 100 lesions per leaf developed after 26 hr of wetness at 15, 22, and 25 C. Disease index analysis showed similar trends in response to temperature and wetness period. A response surface (Fig. 3) was derived from the data to predict disease and illustrate the relationship of temperature (5–25 C) and wetness periods (12–48 hr). Data were extrapolated for temperature (C)/wetness periods (hr) greater than 25/20 and 15/28. Isolation from leaf lesions resulted in a greater than 95% recovery of *W. carpophilus* in all growth chamber studies.

In field experiments, the number of lesions per leaf increased from 0.6 (average for three replications of three cultivars and two experiments) with a 6-hr wetness period treatment to 15.3 with a 16-hr wetness period treatment. Figure 4 shows the effect of duration of the wetness period on the average number of lesions per leaf ($R^2 = 0.89$). Average daily leaf temperatures during the wetness periods ranged from 11.6 to 25.3 C in these experiments. *W. carpophilus* was isolated from 70 to 80% of the lesions that developed during these studies. Other fungal genera that were isolated included: *Alternaria, Cladosporium*, and *Penicillium*.

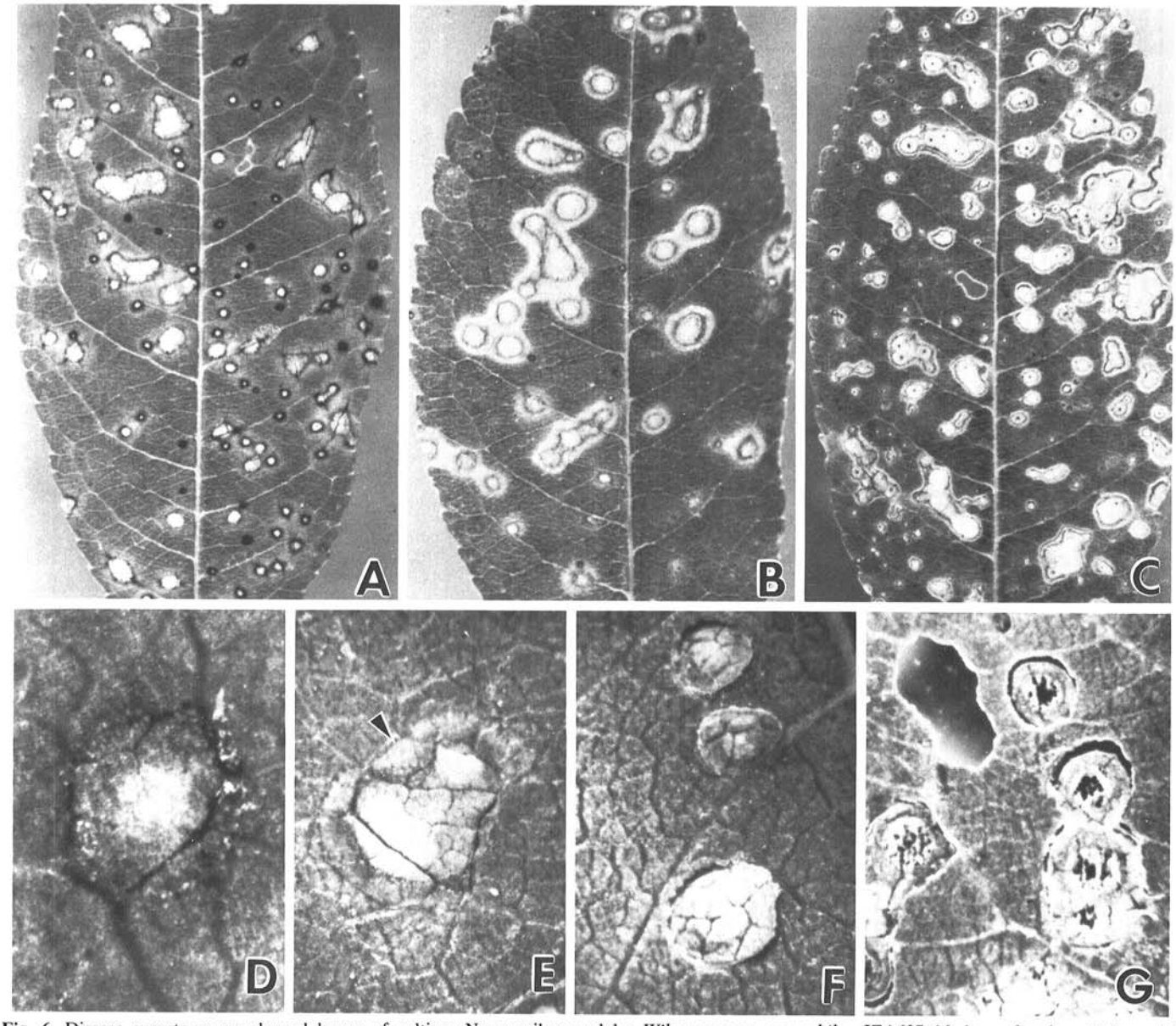


**Fig. 6.** Disease symptoms on almond leaves of cultivar Nonpareil caused by *Wilsonomyces carpophilus* JEA637 16 days after inoculation with conidia and a 16-hr wetness period at 15 C. **A–C and G,** Disease symptoms at 8, 15, and 22 C after four 12-hr wetness periods in 8 days (X2) **A,** Lesions at 8 C with purple margins (dark) and tan-brown centers. **B,** Lesions at 15 C were light brown with a chlorotic halo. **C,** Lesions at 22 C were tan and lacked a halo. Lesions had sporodochia and showed abscission with typical shot hole symptoms. **D–F,** Disease symptoms at 8, 15, and 22 C with no additional wetness periods (×10). **D,** Lesion at 8 C was purple with no distinct border. **E,** Lesions at 15 C with distinct chlorotic halo (arrow). **F,** Lesions at 22 C with abscission. **G,** Lesions at 22 C with abscission and numerous sporodochia.

**Influence of temperature during incubation.** In growth chamber studies, postinoculation temperatures influenced the rate of lesion development and symptom expression on almond leaves. The influence of incubation temperature after the inoculation period on the number of lesions was not significant ($P > 0.05$) in the composite, response surface and the split plot experiments. Decreasing postinoculation temperatures delayed the development of lesions. Inoculated trees were removed from chambers after a 16-hr wetness period at 15 C and incubated at 22 C. Lesions began to develop as small gray flecks after incubation at 22 C for 1 day, 15 C for 2 days, or 8 C for 3 days. After a 5-day incubation period, there were no significant differences ($P > 0.05$) in the numbers of lesions that developed at different temperatures (Fig. 5). Symptom expression also varied among postinoculation temperature treatments (Fig. 6D–G). Lesions were purplish with tan-brown centers at 8 C; lesions were light brown with a chlorotic halo and lacked purple color at 15 C; and light brown with little or no chlorotic halo or purple margin at 22 C.

**Influence of alternating wetting and drying on germination of conidia.** Germination and germ tube elongation occurred after each of five wetting and drying cycles. Length of germination tubes after three cycles is shown in Figure 7. During the initial wetness period, one or more cells of a conidium germinated and germ tubes elongated. Once dried, germination and germ tube elongation ceased and conidia and germ tubes collapsed. In the second and third wetting cycles, conidia rehydrated and germ tubes continued to grow and branch, while some other cells of conidia produced germ tubes that began to elongate. Hyphae developed septa and appressorialike structures within 8 hr of wetness period.

**Influence of alternating wetting and drying on disease development.** In the growth chamber, additional wetness period treatments influenced symptom expression and number of lesions. After 8 days, the disease index in the alternating wet day/dry day treatment ("4") was higher than in the other wetness period treatments at all temperatures and was significantly higher at 8 C (Fig. 8). Average number of lesions/leaf increased significantly ($P \leq 0.05$) from 23.2 in the no additional wetness period treatment ("0") to 54.8 in the alternating wet day/dry day treatment ("4") at 8 C. However, at 15 and 22 C there were no significant differences in average lesions per leaf among wetness period treatments (Fig. 9). In field studies, significant increases in numbers of lesions per leaf and disease indices also resulted from alternating wet/dry treatments where average temperatures were 25 C during the day and 15 C at night. In the zero ("0"), two ("2"), and four ("4") alternating wet/dry treatments, average number of lesions per leaf were 12.6 a, 20.0 ab, and 30.9 b, while average disease indices were 1.6 a, 1.7 a, and 2.4 b, respectively. (Means followed by the same letter were not significantly different using Duncan's multiple range test, $P > 0.05$.)

Percentage of lesions with sporodochia increased after 14 days in the alternating wet day/dry day treatment ("4") at 15 and 22 C (Figs. 6A–C, 10). This treatment resulted in 23 and 36% of the lesions forming sporodochia at 15 and 22 C, respectively. At 22 C significantly more sporodochia formed under the every fourth-day wetness period treatment ("2") than under the no additional wetness period treatment ("0"). Lesion abscission (shot-hole) occurred predominantly at 22 C with 72–76% of the lesions abscised after 16 days regardless of the wetness period treatment (Fig. 11). Fewer than 10% of the lesions abscised at 15 C and <2% at 8 C. Wetness treatment was not a significant factor for lesion abscission at any temperature studied.

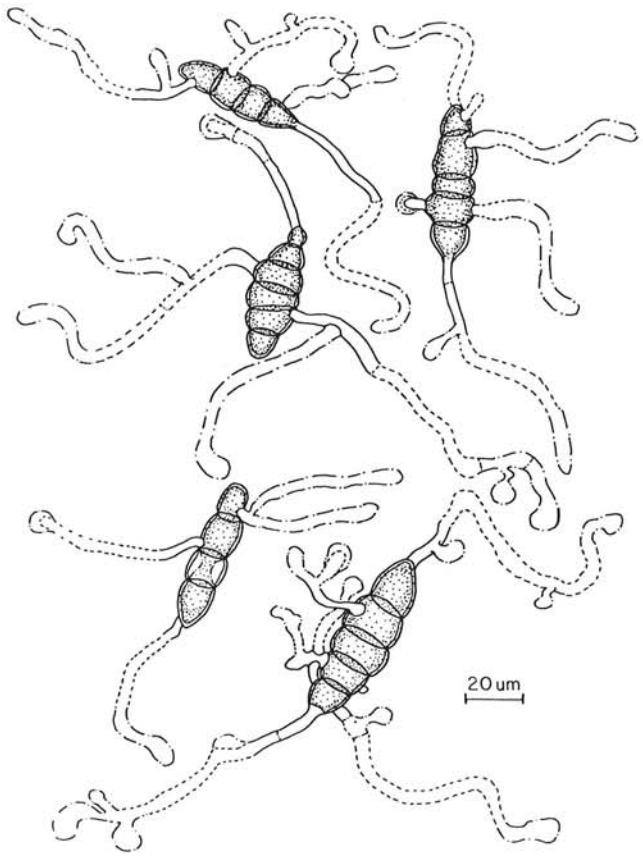


**Fig. 7.** Germination and germ tube elongation of conidia of *Wilsonomyces carpophilus* JEA637 after three alternating wet (4 hr) and dry (20 hr) treatments. Solid lines show growth after 4 hr; dashed lines show growth after the second 4-hr wet treatment (28 hr total) and intermittent dash-dot lines show growth after the third 4-hr wet treatment (52 hr total). No measurable growth occurred during the dry treatments.

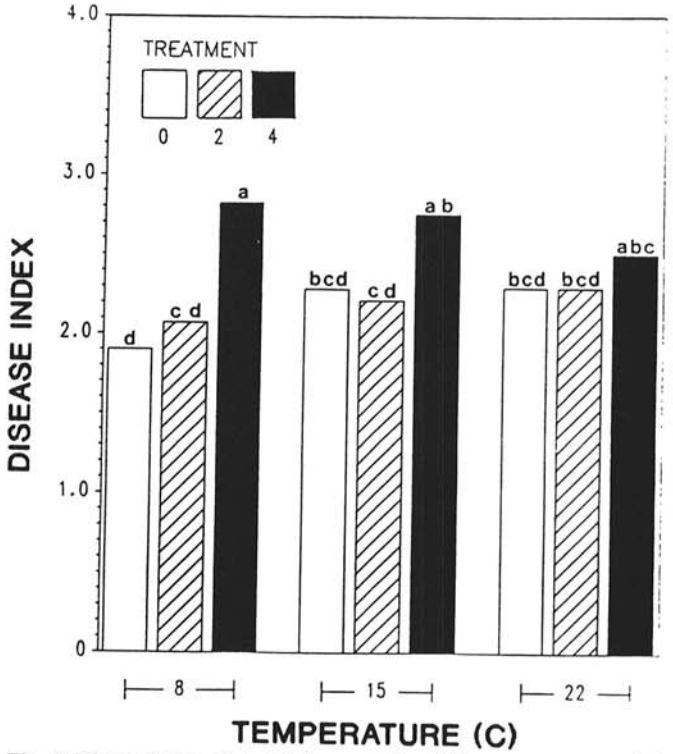


**Fig. 8.** Disease index of shot-hole caused by *Wilsonomyces carpophilus* JEA637 on leaves of cultivars Ne Plus Ultra and Nonpareil 8 days after inoculation and incubation under three wetness regimes at 8, 15, or 22 C. Trees were inoculated with conidia, given a 16-hr wetness treatment at 15 C, and incubated at 8, 15, and 22 C. Alternating wet/dry treatments were: 0 = no additional wetness; 2 = two additional 12-hr wetness treatments over an 8-day period (treated every fourth day); 4 = four additional 12-hr wetness treatments over an 8-day period (treated every other day). Values represent average for all postinoculation wetness treatments. Bars with the same letter are not significantly different using Duncan's multiple-range test ($P \leq 0.05$).

## DISCUSSION

Our results indicate that length of wetness period for infection of almond leaves by *W. carpophilus* depends on temperature (5–25 C). At lower temperatures, longer periods of wetness were required for infection than at high temperatures. At high temperatures, as the wetness period duration increased, a large increase in the numbers of lesions per leaf resulted. Bulger et al (7) indicated that infections of flowers and fruits of strawberry by *Botrytis cinerea* Pers. increased with increasing wetness duration at temperatures between 5 and 30 C in controlled environmental studies. They indicated, however, that flower and fruit infections were greatly reduced above 25 C and below 15 C for all wetness durations up to 32 hr. A similar response at the lower temperatures was observed in our studies with *W. carpophilus* on almond leaves.

In field studies, at daily temperatures ranging from 11.6 to 25.3 C, number of lesions increased with wetness periods longer than 6 hr with more than seven lesions per leaf occurring after wetness periods ≥10 hr. These results agree with our growth chamber studies and our field studies that used the mist generator (2).

Temperature after inoculation period significantly affected the rate of lesion development and symptom expression but did not affect the number of lesions that developed after 5 days. Lesion development was slower at cooler temperatures. This concurs with reported in vitro mycelial growth studies (13,16,17,19,20,22) and suggests that *W. carpophilus* has no dormant period in its infection process of almond leaves. Tomerlin and Jones (21) have shown the presence of a dormant period between infection and symptom expression for *Venturia inaequalis* (Cke.) Wint. Temperature (14,21) and relative humidity (21) influenced the length of the latent period after penetration. In our growth chamber studies, shot-hole lesions on almond leaves developed in 1–4 days at temperatures from 22 to 8 C, respectively, after a 1-day inoculation period with conidia of *W. carpophilus*. Under growth chamber conditions at 22 C, lesions developed as small gray flecks 1 day after incubation and as circular, tan-brown lesions after 3 days of incubation. In field studies, symptoms were observed 5 days after inoculation at 20–22 C (Adaskaveg, *personal observation*). Wilson (23,24) indicated that the incubation period for shot-hole disease at 21 C was 5 to 6 days under field conditions. The reason for the difference between results with trees in the field and potted trees in growth chamber studies is not known. Perhaps there are differences in susceptibility between plant ages, or perhaps the disease develops in a shorter incubation period under constant environmental conditions in controlled studies.

The number of lesions and disease indices significantly increased with additional wetness period in alternating wet/dry treatments in growth chamber studies at 8 C. We observed that with additional wetness period treatments, new lesions developed and existing lesions enlarged and coalesced at 15 and 22 C. Significant differences in number of lesions and disease indices, however, were not detected at these temperatures. The lack of significance in number of lesions and disease indices at higher temperatures may have been due to high inoculum densities, the coalescing of lesions that occurred with the additional wetness period treatments, or the higher rate of disease development at 15 and 22 C which prevented separation of treatments using our schedule for evaluating disease. Our in vitro studies with conidia exposed to wet and dry cycles supports the possibility that short, recurring wetness periods could be conducive to new leaf infections. Upon rehydration, germination of conidia and germ tube elongation continued for at least five 24-hr wet/dry cycles. In contrast, Keitt et al (11) found that conidia of *Blumeriella jaapii* (Rehm) Arx did not survive 12-hr dry periods after germination had occurred.

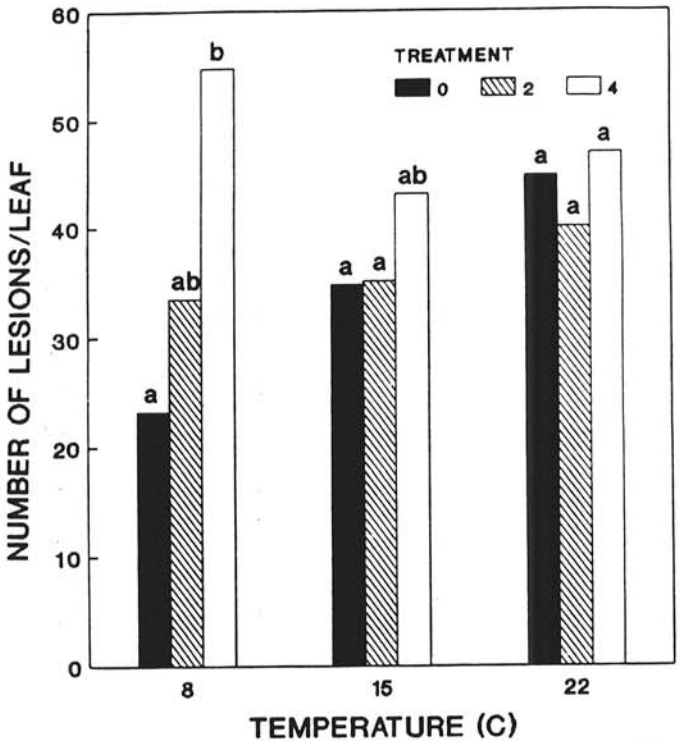


**Fig. 9.** Number of shot-hole lesions caused by *Wilsonomyces carpophilus* JEA637 on leaves of cultivars Ne Plus Ultra and Nonpareil 5 days after inoculation and incubation under three wetness regimes at 8, 15, or 22 C. Trees were inoculated with conidia and given a 16-hr wetness treatment at 15 C. Alternating wet/dry treatments were: 0 = no additional wetness; 2 = two additional 12-hr wetness treatments over an 8-day period (treated every fourth day); 4 = four additional 12-hr wetness treatments over an 8-day period (treated every other day). Values represent average for all postinoculation wetness treatments. Bars with the same letter are not significantly different using Duncan's multiple-range test ($P \leq 0.05$).

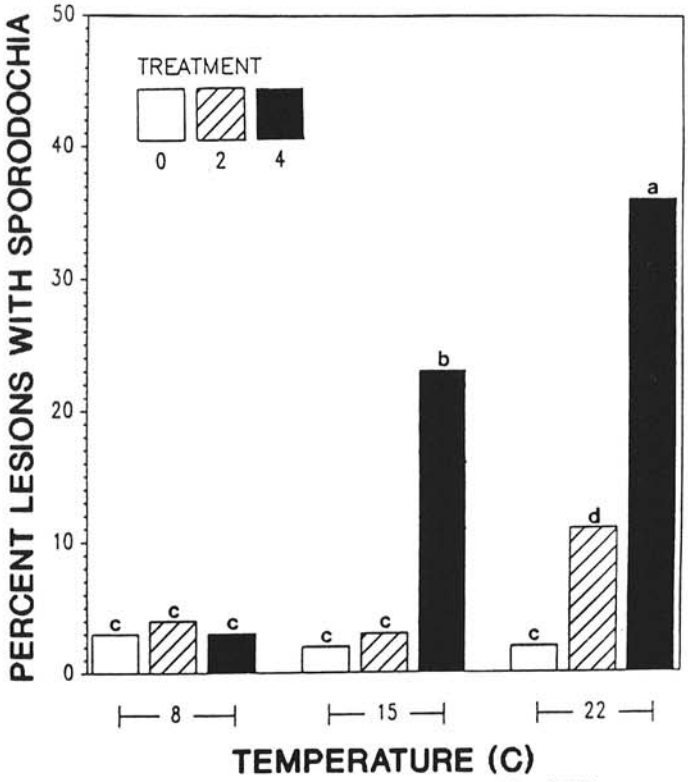


**Fig. 10.** Percentage of shot-hole lesions with sporodochia of *Wilsonomyces carpophilus* on leaves of cultivars Ne Plus Ultra and Nonpareil 14 days after inoculation and incubation under three wetness regimes at 8, 15, or 22 C. Trees were inoculated with conidia of isolate JEA637 and given a 16-hr wetness treatment at 15 C. Alternating wet/dry treatments were: 0 = no additional wetness; 2 = two additional 12-hr wetness treatments over an 8-day period (treated every fourth day); 4 = four additional 12-hr wetness treatments over an 8-day period (treated every other day). Values represent average for all postinoculation wetness treatments. Bars with the same letter are not significantly different using Duncan's multiple-range test ($P \leq 0.05$).

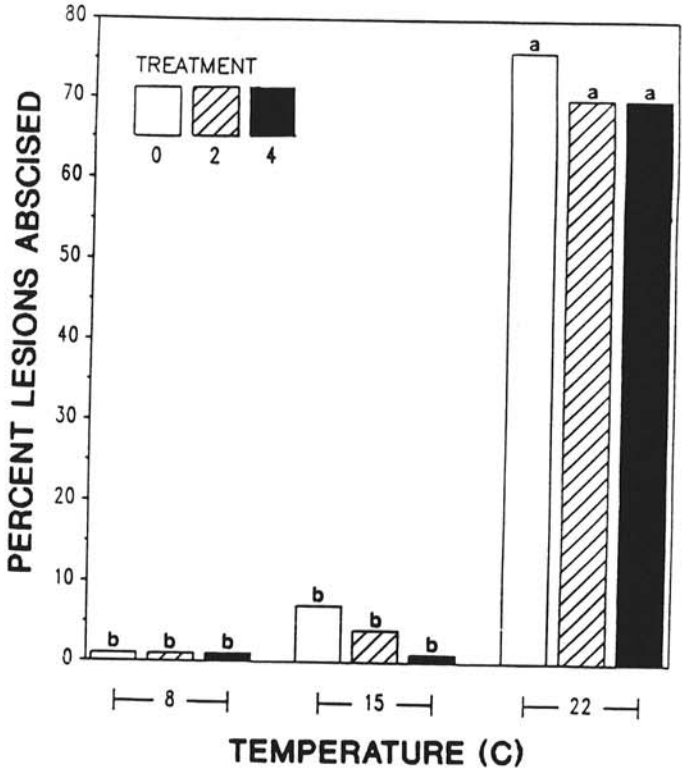


**Fig. 11.** Percentage of shot-hole lesions caused by *Wilsonomyces carpophilus* abscised from leaves of cultivars Ne Plus Ultra and Nonpareil 14 days after inoculation and incubation under three wetness regimes at 8, 15, or 22 C. Trees were inoculated with conidia of isolate JEA637 and given a 16-hr wetness treatment at 15 C. Additional wetness treatments were: 0 = no additional wetness; 2 = two additional 12-hr wetness treatments over an 8-day period (treated every fourth day); 4 = four additional 12-hr wetness treatments over an 8-day period (treated every other day). Bars with the same letter are not significantly different using Duncan's multiple-range test ($P \leq 0.05$).

Formation of sporodochia in lesions increased with additional wetness in the alternating wet/dry treatments ("2" and "4") at 15 and 22 C but not at 8 C in 14 days. Keitt et al (11) found that *B. jaapii* sporulated (conidial stage) at 12–22 C and that sporulation ceased at 28 C. In chamber studies, we found that in 14 days sporodochia of *W. carpophilus* were produced in 36% of the lesions at 22 C with four alternating wet day/dry day treatments ("4") in 8 days. We observed, however, that sporodochia developed with additional wetness in the alternating wet/dry treatments at the cooler temperatures over a longer period of time (approximately 30 days). In the field, sporodochia have been observed in leaf infections that occurred in late fall and early winter months (9).

Lesion abscission occurred at high temperature (22 C) and was independent of additional wetness period treatments in the chamber studies. In the field, the shot-hole symptoms are usually observed in the spring as temperatures increase and shoot growth is active (23). Samuel (18) indicated that abscission occurs between diseased tissue and lignified cells in healthy tissue around the margin of the shot-hole and lesion shrinkage or subsequent leaf expansion causes the lesion to drop out. In our studies, almond leaves responded at high temperatures (22 C) to infection by producing an abscission layer in 16 days. However, this response was limited or absent at cooler temperatures. Similarly, Keitt et al (11) indicated that cooler temperatures (12–20 C) favored sporulation of *B. jaapii* with minimum leaf necrosis, while high temperatures favored necrosis and lesion abscission.

A preliminary model was developed from our studies on the influence of temperature and wetness period on infection of almond leaves by *W. carpophilus* when 300–400 conidia/cm$^2$ of leaf tissue were deposited. The equation defined the effect of the environmental variables temperature and hours of wetness during inoculation on the number of lesions that developed per leaf. The model, containing linear and quadratic components, is similar to that developed for *B. jaapii* on sour cherry (8). Their model, however, predicted an environmental favorability index that was then related to disease incidence. Our model can be used to predict leaf infection between 5 and 25 C when inoculum is present. This temperature range is realistic for conidial germination and mycelial growth of *W. carpophilus* under spring temperatures in California. Further, information was obtained on wetness and temperature affecting disease symptoms and fungal sporulation. This information could be incorporated into a our model to predict disease severity and potential of conidial production. Eventually, control practices may be implemented based on monitored inoculum levels and environmental conditions.

An ideal disease management system includes information about the host, pathogen, and environment (12). Other variables that could be evaluated for our model include: inoculum levels of *W. carpophilus*, survival of conidia under field conditions, effects of relative humidity, wind speed, and other environmental parameters, and effects of all parameters on various tissues and stages of growth of almond.

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