probably are not introduced or deposited inside the larval galleries. This study also shows the favorable effect of cluster compactness on *B. cinerea* infection according to Vail and Marois (22). In both normal and thinned (in which compactness is reduced by half) Sauvignon clusters, the transport of *B. cinerea* conidia by the larvae increased disease incidence. Thus the larvae, when contaminated by conidia, were an efficient vector of *B. cinerea* even if the conditions were less favorable for infection of berries, as in thinned clusters. Therefore, it seems clear that natural contamination of second-generation larvae by *B. cinerea*, as previously reported in the Bordeaux vineyards (8), plays an important role in the epidemiology of the disease. Transport of conidia can partially account for the correlation between the number of second-generation larvae and the number of early centers of Botrytis rot (7).

With respect to damage to ripe berries by third-generation larvae, the effect of contamination with viable conidia was also of importance in our study. The artificial contamination of the larvae with dead conidia led to a 2.3% increase in disease severity at harvest. Because dead conidia occupied the cuticle ornamentations, external contamination with viable vineyard conidia was reduced. Therefore, the increase probably resulted from the infection of damaged berries with wind-dispersed vineyard conidia not being borne on the larvae. An almost twofold increase (+4.7%) in disease severity resulted from inoculation with larvae contaminated with viable conidia. This demonstrates the role of transport of viable conidia in the increased severity of gray mold caused by the larvae of *L. botrana*.

Our findings suggest that grape berry moth larvae can vector *B. cinerea* from infected to healthy grape berries. Therefore, sanitation practices to reduce gray mold in vineyards should include an effective control of the grape berry moth.

LITERATURE CITED

1. Agrios, G. N. 1980. Insect involvement in the transmission of fungal pathogens. Pages 293-324 in: Vectors of Plant Pathogens. K. F. Harris and K. Maramorosch, eds. Academic Press, New York.
2. Ashworth, L. J., Jr., Rice, R. E., McMeans, J. L., and Brown, C. M. 1971. The relationship of insects to infection of cotton bolls by *Aspergillus flavus*. Phytopathology 61:488-493.
3. Brunelli, A., Deseo, K. V., and Malucelli, G. 1978. Ricerche sulla biologia e prove di lotta contro la tignoletta dell'uva (*Lobesia botrana* Den. & Schiff., Lepidoptera, Tortricidae). Atti Giornate Fitopat. 531-538.
4. Bulit, J., and Verdu, D. 1973. Annual and daily variation in the aerial sporing of *Botrytis cinerea* Pers. in a vineyard. Ann. Phytopathol. 5:319.
5. Dubourdieu, D., Pucheu-Planté, B., Mercier, M., and Ribéreau-Gayon, P. 1978. Structure, role et localisation du glucane exocellulaire sécrété par *Botrytis cinerea* dans la baie de raisin. C. R. Acad. Sci. Paris 287:571-573.
6. Eichhorn, K. W., and Lorenz, D. H. 1977. Phänologische Entwicklungsstadien der Rebe. Nachrichtenbl. Dtsch. Pflanzenschutzdienstes (Braunschweig) 29:119-120.
7. Fermaud, M. 1990. Incidence des attaques larvaires d'Eudémis (*Lobesia botrana* Den. & Schiff.) sur le développement de la Pourriture grise (*Botrytis cinerea* Pers.) chez la vigne. Ph.D. thesis. Institut National Agronomique Paris-Grignon, France. 104 pp.
8. Fermaud, M., and Le Menn, R. 1989. Association of *Botrytis cinerea* with grape berry moth larvae. Phytopathology 79:651-656.
9. Galet, P. 1977. Les Maladies et les Parasites de la Vigne, tome I. Imprimerie du "Paysan du Midi," Montpellier. 871 pp.
10. Hill, G., Stellwaag-Kittler, F., Huth, G., and Schlösser E. 1981. Resistance of grapes in different developmental stages to *Botrytis cinerea*. Phytopathol. Z. 102:328-338.
11. Huglin, P. 1986. Biologie et Écologie de la Vigne. Editions Payot, Lausanne. 372 pp.
12. Jarvis, W. R. 1977. *Botryotinia* and *Botrytis* species: Taxonomy, physiology and pathogenicity. Monogr. 15. Can. Dep. Agric.
13. Lee, L. S., Lacey, P. E., and Goynes, W. R. 1987. Aflatoxin in Arizona cottonseed: A model study of insect-vectored entry of cotton bolls by *Aspergillus flavus*. Plant Dis. 71:997-1001.
14. Mc Clellan, W. D., and Hewitt, W. B. 1973. Early Botrytis rot of grapes: Time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. Phytopathology 63:1151-1157.
15. Remund, U., and Siegfried, W. 1982. Zur Sauerwurm-*Botrytis*-Beziehung. Schweiz. Z. Obst Weinbau 118:277-285.
16. Ribéreau-Gayon, J., Ribéreau-Gayon, P., and Seguin, G. 1980. *Botrytis cinerea* in enology. Pages 251-274 in: The Biology of *Botrytis*. J. R. Coley-Smith, K. Verhoeff, and W. R. Jarvis, eds. Academic Press, New York. 318 pp.
17. SAS Institute. 1988. SAS User's Guide: Statistics. Release 6.03 edition. SAS Institute, Cary, NC.
18. Schmid, A., and Antonin, P. 1977. *Bacillus thuringiensis* dans la lutte contre les vers de la grappe, eudémis (*Lobesia botrana*) et cochylis (*Clysia ambiguella*) en Suisse romande. Rev. Suisse Vitic. Arboric. Hortic. 9:119-126.
19. Snedecor, G. W., and Cochran, W. G. 1957. Statistical Methods. Iowa State University Press, Ames.
20. STAT-ITCF. 1988. 4ème Version. Manuel d'utilisation. ITCF, Paris.
21. Stockel, J., Roehrich, R., Carles, J. P., and Nadaud, A. 1989. Technique d'élevage pour l'obtention programmée d'adultes vierges d'Eudémis. Phytoma 412:45-47.
22. Vail, M. E., and Marois, J. J. 1991. Grape cluster architecture and the susceptibility of berries to *Botrytis cinerea*. Phytopathology 81:188-191.
23. Valli, G. 1975. Lotta integrata nei vigneti. Ricerche e valutazioni preliminari sulle Tignole. Not. Mal. Piante 92/93:407-419.
24. Velimirovic, V. 1976. Contribution to the study of the ashen vine moth *Lobesia botrana* (Lepidoptera, Tortricidae) in the region of Crna Gora. Rev. Appl. Entomol. Ser. A. 64:5548.
25. Voigt, E. 1972. Damages in vineyards of Hungary caused by *Argyrotaenia pulchellana* Haw. Pflanzenschutzberichte 43:13-23.
26. Webber, J. F., and Gibbs, J. N. 1989. Insect dissemination of fungal pathogens of trees. Pages 161-189 in: Insect–Fungus Interactions. N. Wilding, N. M. Collins, P. M. Hammond, and J. F. Webber, eds. Academic Press, London.

Effects of Temperature and Wetness Duration on Infection of Pistachio by *Botryosphaeria dothidea* and Management of Disease by Reducing Duration of Irrigation

Themis J. Michailides and David P. Morgan

Associate research plant pathologist and staff research associate, respectively, University of California, Berkeley/Kearney Agricultural Center, 9240 S. Riverbend Ave., Parlier, CA 93648.

We thank F. & M. Montgomery (Almond Orchards, Durham, CA), J. Bradford (Ballards Orchards Corporation, Vina, CA), and R. Schrum for their cooperation and permission to manipulate irrigation duration in their orchards, and J. Grant and W. Olson (University of California Cooperative Extension) for assistance in the field.

This research was supported by funds from the California Pistachio Commission.

Accepted for publication 21 July 1992.

ABSTRACT

Michailides, T. J., and Morgan, D. P. 1992. Effects of temperature and wetness duration on infection of pistachio by *Botryosphaeria dothidea* and management of disease by reducing duration of irrigation. Phytopathology 82:1399-1406.

The optimum temperatures for growth of *Botryosphaeria dothidea*, the cause of Botryosphaeria panicle and shoot blight of pistachio, were 27–30 C on potato-dextrose agar. The optimum temperatures for pycnidiospore germination were 24–36 C. Pycnidiospores that germinated at 36–39 C failed to develop colonies. Optimum temperatures for disease development on artificially inoculated fruit were 27–33 C and for development of pycnidial initials and pycnidia on fruit, 30 C. The latent period for pycnidia development on pistachio fruit naturally infected by *B. dothidea* was shorter at 33 C than at 10–27 C. Pycnidia did not develop at 6 C and formed on only about 17% of the fruit at 10 C. Field-inoculated pistachio leaves kept wet continuously for 0–72 h developed no disease at wetness durations of 0–6 h; durations of 9–12 h or longer resulted in symptoms and increased disease severity. Ninety-seven and 100% of the inoculated leaves had lesions, approximately 25 and 48% of them showing more than 10 lesions per leaf after 48 and 72 h of wetness duration, respectively. Disease incidence of leaves was best described by a third-order polynomial regression equation. Cluster blight, fruit with pycnidia, and leaf (0.4–6.0%) and petiole blight (15–84.4%) occurred only after 18 h of wetness and increased linearly with wetness durations of 18–72 h. In field experiments, two to four additional 12-h wetness periods after an initial 12-h infection period interrupted by 12-h dry periods resulted in greater disease incidence and severity on leaves. Reducing duration of sprinkler irrigation from 24 to 12 h in one orchard in Butte County resulted in reduction of the disease (infected fruit) from 31 to 20% ($P = 0.10$), from 60 to 48% ($P = 0.08$), and from 62 to 19% ($P < 0.01$) in 1989, 1990, and 1991, respectively. In addition, reducing duration of sprinkler irrigation in the same orchard to 12 h resulted in significantly lower incidence and severity of infected leaves compared with leaves from trees irrigated for 24 h. Similarly, in 1991 in a second orchard, reduction of duration of sprinkler irrigation from 48 to 24 h significantly lowered the incidence and the severity of leaves infected by *B. dothidea*.

Additional keywords: *Botryosphaeria ribis*, *Dothiorella* sp., *Pistacia vera*, quantitative epidemiology.

Botryosphaeria panicle and shoot blight of pistachio (*Pistacia vera* L.), caused by a *Dothiorella* sp. of the teleomorph *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not., is a common disease in orchards in northern California, where it causes significant yield losses because it kills fruit panicles. To date, the fungus has only been isolated from trees in pistachio orchards in central and southern California (12). Sources of inoculum are pycnidia developed on retained rachises and petioles and on cankers of blighted shoots and panicles (12).

Various tests conducted in vitro and in vivo to produce the perfect stage of *B. dothidea* from pistachio had no success (T. J. Michailides, *unpublished*). Infrequent occurrence of the sexual stage of this fungus in infected host tissue and/or in culture media has been reported previously (10,20,24,25). Although ascocarps of *B. dothidea* have been discovered in giant sequoia (*Sequoiadendron giganteum*) and coast redwood (*Sequoia sempervirens*) in California (26), they have not yet been found in any of the pistachio orchards that we surveyed, where Botryosphaeria panicle and shoot blight is a problem (T. J. Michailides, *unpublished*). Because no ascocarps have been found in pistachio orchards, disease outbreaks apparently depend on pycnidiospores present in the orchard, the rates of pycnidiospore spread, and environmental conditions.

Pycnidiospores of the fungus that are spread by rain contaminate vegetative and flower buds during fall or winter and cause subclinical infections (16). During the spring, rainfall and increasing temperatures provide conditions favorable for primary infection of the developing shoots and blossoms, resulting in shoot and panicle blights, respectively. Secondary infections by the fungus may occur in late summer and fall as a result of sprinkler irrigation or early fall rainfall. Because mature pycnidiospores are present by mid-August in most of the infected plant parts (12), water droplets can disperse the fungal spores and/or provide a film of wetness for infection. Multiple infections result in severe defoliation of the trees in some years, and partial or total panicle blight (16). Infections of buds during summer and/or fall result in bud blight and partial bud blight, the latter resulting in shoot or blossom blight in the following growing season (16). Pycnidiospores from current season pycnidia, as well as pycnidiospores from 1- or 2-yr-old pycnidia found in cankered wood, can provide inoculum for primary spring and summer infections (12).

Outbreaks of Botryosphaeria blight occurred in several orchards irrigated by sprinklers, especially in those with a high trajectory nozzle angle sufficient to wet fruit panicles and foliage on the lower tree branches. In contrast, nearby flood-irrigated pistachio orchards were essentially devoid of the disease. Under conditions of temperature and wetness that favor disease during spring and summer, yield loss can be significant. However, control of the disease in an orchard in 1985 resulted in an estimated 40% increase of yield (16,17). The majority of pistachio plantings are located in central California (4), where Botryosphaeria panicle and shoot blight is not considered a serious problem. In sprinkler-irrigated orchards in northern California, however, the disease can become severe in late spring and summer.

The only fungicide currently registered in California for the control of Botryosphaeria panicle and shoot blight is benomyl (Benlate 50 DF) applied once during full bloom (5,16). But even

wet by opening the bags and rewetting the shoots and leaves every 6–10 h as necessary. Shoots were covered for 3, 6, 9, 12, 15, 18, 24, 48, and 72 h. Wetness duration included the time required for leaves and clusters to dry after removal of the bags. Drying times and continuous wetness durations were determined visually (when opening the bags to rewet the shoots) and with a leaf wetness sensing grid (Model 237) connected to a 21× datalogger (both made by Campbell Scientific). Shoots inoculated as described but not covered with polyethylene and paper bags served as a treatment of 0 h of wetness duration. Five shoots were used for each duration treatment. Twenty to 25 days after inoculation, we determined disease severity by counting lesions on the leaf blades and classifying the leaves into five (0–4) severity categories: 0 = leaves with no infection lesions, 1 = one to five lesions per leaf, 2 = six to 10, 3 = 11–15, and 4 = ≥16. Disease index (DI) was determined as

$$DI = \frac{A \times 0 + B \times 1 + C \times 2 + D \times 3 + E \times 4}{A + B + C + D + E} \quad (1)$$

in which A, B, C, D, and E = number of leaves in 0, 1, 2, 3, and 4 severity categories, respectively.

In addition, the numbers of blighted leaf blades, petioles, clusters, fruits, fruits bearing mature pycnidia, and blighted shoots were determined. The experiment was repeated once, and results were averaged, because results from the two repetitions were virtually the same ($F = 1.1$; $P > 0.05$).

Effects of interrupted wetness periods on disease incidence and severity. Fifteen to 20 mature leaves on each of 20 shoots of (cv. Kerman) pistachios were sprayed to run-off with 10^5 spores per milliliter of *B. dothidea* at 0700 h and covered immediately as described. At 1900 h, polyethylene and paper bags were removed from all shoots. After a 12-h drying period, 15 of the 20 inoculated shoots were sprayed with water; a hand-held sprayer was used. Then the shoots were covered with polyethylene and paper bags for 12 h. This process was repeated four times so that five shoots each were subjected to wetness periods of 12, 12 + 12, 12 + 12 + 12, and 12 + 12 + 12 + 12 h interrupted by drying periods of 12 h. Twenty shoots, sprayed with distilled water and wetted in a manner similar to the inoculated shoots, served as controls. A temperature probe (Model 108) and a leaf wetness sensing grid (Model 237) connected to a datalogger (Model 21×) (all made by Campbell Scientific) were sprayed with distilled water, covered with polyethylene and paper bags for 12 h, and allowed to dry for 12 h for four repeated cycles; they were used to monitor temperature and RH ambient and inside the polyethylene bags during these experiments. Incidence and severity of the disease were recorded 15 days after the last wetting period. Because similar trends were found in the second repetition of this experiment, only results from the first experiment are presented.

Control of disease by reducing duration of irrigation (experiments in 1989, 1990, and 1991). Because it was suspected that irrigations of long duration (24–48 h) had contributed to a severe outbreak of disease in an orchard near Durham in Butte County, where inoculum of *B. dothidea* was distributed uniformly throughout the orchard, an experiment was designed to determine the effects of different irrigation durations in reducing disease incidence and severity. The experiment was established in a 1.3-ha 30-yr-old pistachio orchard in a completely randomized block design with three 42-tree replications (Fig. 1). The plot was irrigated with low-pressure impact sprinklers (of 12° trajectory angle) provided with diffuser nozzles with 3.6-mm orifices (Nelson 2DN Diffuser Nozzles, Nelson Irrigation Co., Walla Walla, WA) operated at a pressure of 2.81 kg/cm^2. There were five sprinklers on the irrigation pipe for each replicated block. The duration of irrigation was controlled by shut-off valves connected to the risers of the five sprinklers in the three replicated blocks to be irrigated for 12 h (Fig. 1). The irrigation pipe remained at each position for 24 h; irrigation started at 0600 and stopped at 1800 h. At the latter time, valves in half of each of the three blocks (three 42-sets of trees) were closed (short duration); the irrigation continued for 12 h in the other half of each of the three blocks (long duration) before the irrigation pipe was moved to a second position (Fig. 1). The irrigation of the experimental plot was completed with three movements of the irrigation pipe within 3 days as shown in the diagrammatic presentation of the plot (Fig. 1).

Disease incidence was recorded on 10 selected and marked fruit clusters per tree on two trees per block at a height of 1.25–1.50 m. During commercial harvest (10–15 September), fruits from marked clusters were harvested and brought to the laboratory. For each experimental tree, subsamples of 200 nuts and 50 leaves were collected randomly and evaluated for disease: incidences of infected fruits, fruits with pycnidia, infected and blighted rachises, and leaves with lesions. We determined leaf disease index by using Equation 1. The experiment was conducted in 1989, 1990, and 1991. Data were analyzed with analysis of variance (ANOVA), and mean differences were compared with LSD. SAS statistics were used.

In 1991, a second experimental plot was established in a 1.0-ha commercial 10-yr-old pistachio (cv. Kerman) orchard near Stockton, CA, in San Joaquin County, where the disease had caused significant yield losses during 1987–1990 (13,14). In this orchard, the sprinklers (Rain Bird L20VL bearing a 2-mm nozzle orifice) (Rain Bird Sales, Inc., Agri-Products Division, Glendora, CA) were permanently set on the ground and connected with underground pipes. There were 30 trees in each replicated block, and seven to eight sprinklers were uniformly distributed. To control the duration of irrigation in the experimental plot, we cut the sprinkler risers and attached a hand shut-off valve in half of the replicated blocks. By shutting off the valves, we kept the duration of irrigation in the three blocks at 24 h (short duration), whereas we continued irrigation for 24 h more in the other three replicated blocks (long duration). Because of the grower's prescheduled fruit harvest, only leaves were evaluated for disease incidence and severity in this experiment. Fifty leaves were ran-

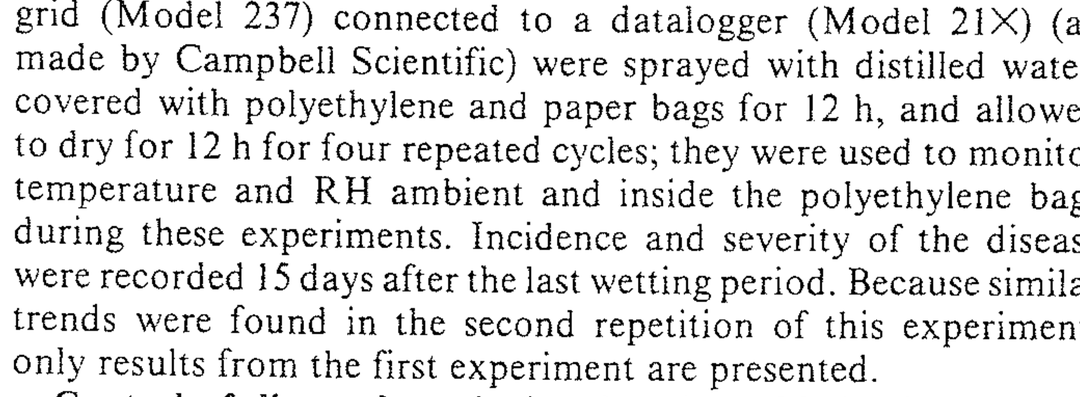

Fig. 1. Partial diagram of experimental plot in the pistachio orchard in Durham, California (Butte County), indicating the set-up of blocks (two replications) irrigated by sprinklers for 12 and 12 + 12 h.

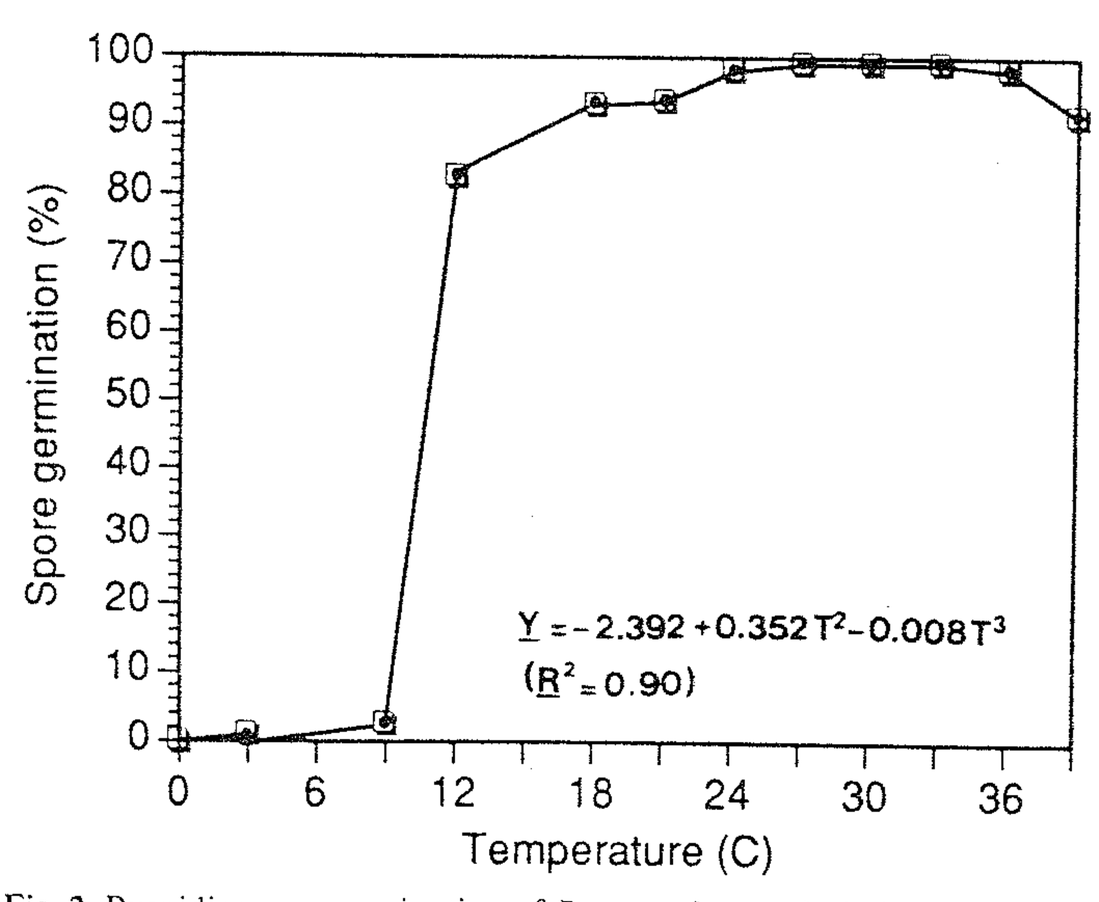

Fig. 2. Pycnidiospore germination of *Botryosphaeria dothidea* at different temperatures. Y = percentage of spore germination and T = temperature (C); significant at $P < 0.0001$.

with application of this fungicide, disease control in several orchards with high levels of inoculum is not satisfactory (14). Current sprinkler irrigation practices in some California pistachio orchards evidently favor disease outbreaks by providing long wetting conditions (24–48 h per irrigation) that tend to result in increased fruit and leaf infections. If the effects of temperature and wetness duration on disease development on various host tissues are more clearly understood, it may be possible to predict disease outbreaks, devise more efficient control strategies by manipulation of irrigation, and significantly reduce losses in pistachio orchards.

No studies exist on the effects of temperature and wetness duration on this disease. The objectives of this study were to determine the effects of temperature and wetness duration on the development of Botryosphaeria panicle and shoot blight of pistachio and to determine if control of the disease by reducing the duration of sprinkler irrigation was feasible. Preliminary reports of parts of this study have been published (13,15).

MATERIALS AND METHODS

Isolates and inoculum production. Isolates of *B. dothidea* were obtained from pistachio fruits and leaves from the oldest commercial pistachio orchard in Butte County, California, where Botryosphaeria panicle and shoot blight was first discovered (21). Two isolates, one from a fruit (Bd-5F) and one from a leaf (Bd-7L), were grown on potato-dextrose agar (PDA) (Difco Laboratories, Detroit, MI) at 25 C under constant light (two 40-W cool-white fluorescent lights 20 cm above the dishes) for 15 days to encourage production of pycnidiospores. These two isolates were used in preliminary experiments to test for pathogenicity and to complete Koch's postulates. Because no significant ($F = 0.04$; $P > 0.05$) differences in growth and pathogenicity were determined between the two isolates, only the isolate Bd-5F was used in inoculation of fruits and leaves of pistachio in the laboratory and the field. Stock cultures were stored in grade 40 silica gel (Davison Chemical, Baltimore, MD) at 9 C.

We collected spore inoculum by washing cultures of *B. dothidea* growing on acidified (2.5 ml of a 25% [v/v] solution of lactic acid per liter of medium) PDA (APDA) or on pistachio fruit decoction agar (185 g of ground green pistachio fruit and 39 g of Difco PDA in a liter of distilled water) with 10–15 ml of sterile distilled water and by scraping the surface of the agar with a rubber policeman. Spore suspensions were filtered through four layers of cheesecloth to eliminate mycelial fragments, adjusted to desired concentrations with a hemacytometer, and used as needed.

Effects of temperature on spore germination and mycelial growth. A 100-μl suspension of 2×10^5 pycnidiospores per milliliter prepared from a 15-day-old culture of each *B. dothidea* isolate (Bd-5F and Bd-7L) was pipetted on 48 dishes (per isolate) containing APDA. Four dishes were incubated at temperatures of 0, 4, 9, 12, 18, 21, 24, 27, 30, 33, 36, and 39 C (±1 C at each temperature) for 7 h. Fifty spores per dish were counted; a spore was considered germinated if its germ tube measured approximately half the spore length. The experiment was repeated once. Regression analysis was performed to determine the effect of temperature on the pycnidiospore germination; the GLM procedure of Statistical Analysis Systems (SAS Institute, Inc., Cary, NC) was used.

Petri dishes containing APDA were inoculated centrally with a 5-mm-diameter mycelial plug from the margins of 4-day-old cultures of *B. dothidea* isolates (Bd-5F and Bd-7L) grown on APDA. Cultures were incubated at 0, 6, 9, 15, 21, 24, 27, 30, 33, 36, and 39 C (±1 C at each temperature), and colony diameter was recorded after 6 days. Because no significant ($P > 0.05$) differences in growth and sporulation were determined between the two isolates, the results were averaged; the experiment was repeated once with essentially similar results.

Effects of temperature on infection and sporulation of *B. dothidea* on pistachio fruits and leaves. Healthy pistachio clusters (cv. Kerman) were collected from two 20-yr-old trees on 15 June from the University of California Wolfskill Experimental Orchards (UC-WEO) at Winters, California. After being surface-disinfected in a 0.08% NaOCl solution and rinsed with sterile distilled water, six clusters of 15–20 fruits each were placed over waxed wire screens (20 × 29.5 cm) in plastic containers (23.5 × 32 × 10 cm) and allowed to dry for 3–6 h. The clusters were then sprayed with a suspension of 10^5 spores per milliliter of *B. dothidea* prepared from a 15-day-old culture on pistachio nut decoction agar; the method described earlier was used. To provide moisture, 250 ml of distilled water was poured on the bottom of each container, providing 98–100% relative humidity (RH) measured with a temperature-RH sensor (Model XN217, Hygrometrix, Inc., Oakland, CA) and recorded by a datalogger (Model 21X, Campbell Scientific, Logan, UT). Containers were incubated for 10 days at 15, 21, 24, 27, 30, 33, 36, and 39 C (±1 C at each temperature). The temperature in each incubator was checked periodically with a min-max thermometer. The percentage of infected fruit was recorded 5 and 10 days after inoculation. Fruits with pycnidia and pycnidial initials were counted 10 days after inoculation. In addition, differences in symptom morphology (color and size of infection lesions) on inoculated fruits at different temperatures were recorded after 10 days of incubation at each temperature. Each experiment was repeated once. Because the experiments did not differ significantly ($F = 1.02$ and $P = 0.31$ for fruit inoculations; and $F = 0.04$ and $P = 0.84$ for leaf inoculations), the results from the two trials were averaged.

In another experiment, healthy leaves were collected from a pistachio tree (cv. Kerman) in mid-May, surface-disinfected by washing them in a 0.08% NaOCl solution for 3–4 min, rinsed with deionized water, and allowed to dry for 3 h at 22 ± 1 C. Eight petiolate leaflets were placed (adaxial surface facing up) on waxed wire screens in plastic containers and sprayed by use of a Vilbriss atomizer with a pycnidiospore suspension (10^5) prepared from *B. dothidea* cultures on pistachio nut decoction agar as previously described. To maintain high relative humidity in the containers, 250–300 ml of deionized water was added per container. Containers were covered and incubated in incubators set at 15, 21, 24, 30, 33, 36, and 39 C (±1 C at each temperature). To maintain continuous wetness periods corresponding to incubation time, we sprayed the leaves in each container every other day with about 5 ml of deionized water; we used a hand atomizer. The leaves were observed for symptom development 2 days after inoculation, and the percentage of infected leaf area was determined 5 and 10 days after inoculation. Pycnidial development was observed for up to 20 days of incubation. There were three replicated containers for each experiment, and data presented are the average of two experiments, because the two experiments did not differ statistically ($P > 0.05$).

Latent periods for pycnidia development at different temperatures on naturally infected fruit. Naturally infected mature pistachio fruit, showing characteristic symptoms but devoid of signs of sporulation, were collected from an orchard in Butte County (Durham, California) that had a high incidence of the disease. Fifty fruits (five 10-fruit replicates) were placed over a waxed screen in a plastic container (23.5 × 32 × 10 cm), containing 250 ml of distilled water incubated at 6, 10, 20, 27, and 33 C (±1 C at each temperature) for 10 days. The numbers of fruits with pycnidia were determined by examining each fruit with a dissecting microscope after 4, 6, and 10 days. The experiment was repeated with infected fruits collected from a second commercial orchard in Tehama County at Vina, California. Data were analyzed by regression; GLM procedures of SAS were used.

Effects of continuous wetness periods on disease incidence and severity. Current season shoots bearing seven to 11 leaves and three to six fruit clusters on pistachio trees (five each of female cv. Kerman and male cv. Peters) each were sprayed to run-off with approximately 10 ml of a suspension of 10^5 spores per milliliter of *B. dothidea* (isolate Bd-5F). Each shoot was immediately covered with a 10-mil polyethylene bag that was sprayed inside with distilled water and secured to the shoot with masking tape. A brown paper bag was placed over the plastic bag to protect shoots and leaves from sunburn. Shoots were kept continuously

wet by opening the bags and rewetting the shoots and leaves every 6–10 h as necessary. Shoots were covered for 3, 6, 9, 12, 15, 18, 24, 48, and 72 h. Wetness duration included the time required for leaves and clusters to dry after removal of the bags. Drying times and continuous wetness durations were determined visually (when opening the bags to rewet the shoots) and with a leaf wetness sensing grid (Model 237) connected to a 21× datalogger (both made by Campbell Scientific). Shoots inoculated as described but not covered with polyethylene and paper bags served as a treatment of 0 h of wetness duration. Five shoots were used for each duration treatment. Twenty to 25 days after inoculation, we determined disease severity by counting lesions on the leaf blades and classifying the leaves into five (0–4) severity categories: 0 = leaves with no infection lesions, 1 = one to five lesions per leaf, 2 = six to 10, 3 = 11–15, and 4 = ≥16. Disease index (DI) was determined as

$$DI = \frac{A \times 0 + B \times 1 + C \times 2 + D \times 3 + E \times 4}{A + B + C + D + E} \quad (1)$$

in which A, B, C, D, and E = number of leaves in 0, 1, 2, 3, and 4 severity categories, respectively.

In addition, the numbers of blighted leaf blades, petioles, clusters, fruits, fruits bearing mature pycnidia, and blighted shoots were determined. The experiment was repeated once, and results were averaged, because results from the two repetitions were virtually the same ($F = 1.1$; $P > 0.05$).

Effects of interrupted wetness periods on disease incidence and severity. Fifteen to 20 mature leaves on each of 20 shoots of (cv. Kerman) pistachios were sprayed to run-off with 10^5 spores per milliliter of *B. dothidea* at 0700 h and covered immediately as described. At 1900 h, polyethylene and paper bags were removed from all shoots. After a 12-h drying period, 15 of the 20 inoculated shoots were sprayed with water; a hand-held sprayer was used. Then the shoots were covered with polyethylene and paper bags for 12 h. This process was repeated four times so that five shoots each were subjected to wetness periods of 12, 12 + 12, 12 + 12 + 12, and 12 + 12 + 12 + 12 h interrupted by drying periods of 12 h. Twenty shoots, sprayed with distilled water and wetted in a manner similar to the inoculated shoots, served as controls. A temperature probe (Model 108) and a leaf wetness sensing grid (Model 237) connected to a datalogger (Model 21×) (all made by Campbell Scientific) were sprayed with distilled water, covered with polyethylene and paper bags for 12 h, and allowed to dry for 12 h for four repeated cycles; they were used to monitor temperature and RH ambient and inside the polyethylene bags during these experiments. Incidence and severity of the disease were recorded 15 days after the last wetting period. Because similar trends were found in the second repetition of this experiment, only results from the first experiment are presented.

Control of disease by reducing duration of irrigation (experiments in 1989, 1990, and 1991). Because it was suspected that irrigations of long duration (24–48 h) had contributed to a severe outbreak of disease in an orchard near Durham in Butte County, where inoculum of *B. dothidea* was distributed uniformly throughout the orchard, an experiment was designed to determine the effects of different irrigation durations in reducing disease incidence and severity. The experiment was established in a 1.3-ha

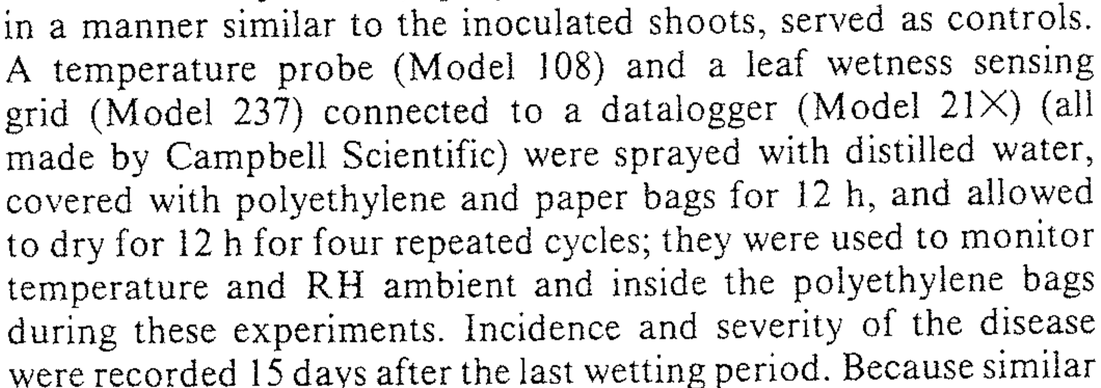

Fig. 1. Partial diagram of experimental plot in the pistachio orchard in Durham, California (Butte County), indicating the set-up of blocks (two replications) irrigated by sprinklers for 12 and 12 + 12 h.

30-yr-old pistachio orchard in a completely randomized bloc design with three 42-tree replications (Fig. 1). The plot was irr gated with low-pressure impact sprinklers (of 12° trajectory angl provided with diffuser nozzles with 3.6-mm orifices (Nelson 2D Diffuser Nozzles, Nelson Irrigation Co., Walla Walla, WA) ope ated at a pressure of 2.81 kg/cm^2. There were five sprinkle on the irrigation pipe for each replicated block. The duratic of irrigation was controlled by shut-off valves connected to th risers of the five sprinklers in the three replicated blocks to b irrigated for 12 h (Fig. 1). The irrigation pipe remained at eac position for 24 h; irrigation started at 0600 and stopped at 1800 At the latter time, valves in half of each of the three blocks (thr 42-sets of trees) were closed (short duration); the irrigatic continued for 12 h in the other half of each of the three bloc (long duration) before the irrigation pipe was moved to a secor position (Fig. 1). The irrigation of the experimental plot w completed with three movements of the irrigation pipe with 3 days as shown in the diagrammatic presentation of the pl (Fig. 1).

Disease incidence was recorded on 10 selected and marked fru clusters per tree on two trees per block at a height of 1.25–1.50 r During commercial harvest (10–15 September), fruits fro marked clusters were harvested and brought to the laborator For each experimental tree, subsamples of 200 nuts and 50 leav were collected randomly and evaluated for disease: incidenc of infected fruits, fruits with pycnidia, infected and blight rachises, and leaves with lesions. We determined leaf disease ind by using Equation 1. The experiment was conducted in 198 1990, and 1991. Data were analyzed with analysis of varian (ANOVA), and mean differences were compared with LSD. SA statistics were used.

In 1991, a second experimental plot was established in 1.0-ha commercial 10-yr-old pistachio (cv. Kerman) orchard ne Stockton, CA, in San Joaquin County, where the disease h caused significant yield losses during 1987–1990 (13,14). In th orchard, the sprinklers (Rain Bird L20VL bearing a 2-mm noz orifice) (Rain Bird Sales, Inc., Agri-Products Division, Glendo CA) were permanently set on the ground and connected wi underground pipes. There were 30 trees in each replicated bloc and seven to eight sprinklers were uniformly distributed. To co trol the duration of irrigation in the experimental plot, we c the sprinkler risers and attached a hand shut-off valve in h of the replicated blocks. By shutting off the valves, we kept t duration of irrigation in the three blocks at 24 h (short duratio whereas we continued irrigation for 24 h more in the other thr replicated blocks (long duration). Because of the grower's p scheduled fruit harvest, only leaves were evaluated for disea incidence and severity in this experiment. Fifty leaves were ra

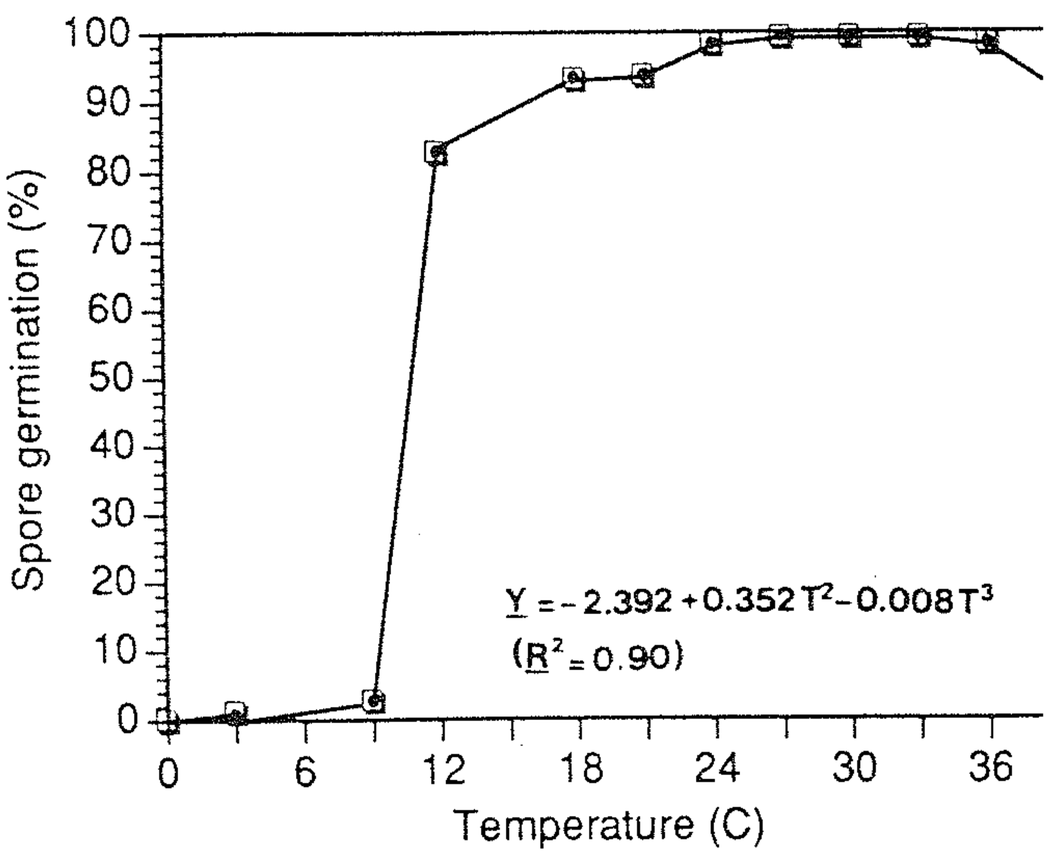

Fig. 2. Pycnidiospore germination of *Botryosphaeria dothidea* at diffe temperatures. Y = percentage of spore germination and T = temperatu (C); significant at $P < 0.0001$.

domly collected from each of four trees in the center of each replicated block of trees and evaluated as previously described. Data were analyzed with ANOVA, and comparison of means was done with LSD by use of SAS statistics.

RESULTS

Isolates. Because no differences ($P > 0.05$) in growth rates, sporulation, and pathogenicity were detected between the two isolates (Bd-5F and Bd-7L) of *B. dothidea* tested, the results were averaged.

Effects of temperature on spore germination and mycelial growth. No pycnidiospores of *B. dothidea* germinated at 0–4 C, only 2% germinated at 9 C, but approximately 80% of the spores germinated at 12 C after 7 h of incubation (Fig. 2). The percentage of germination increased with temperature; the optimum temperatures for pycnidiospore germination were 24–36 C (Fig. 2). At 36 and 39 C, pycnidiospores germinated, but colonies failed to develop.

Mycelial growth of *B. dothidea* was influenced by temperature. *B. dothidea* grew optimally at 27–30 C and failed to grow at 0–6 C and 36–39 C, even after 110 days of incubation (Fig. 3). However, colonies of 10 mm in diameter were formed after 120 days of incubation at 6 C.

Effects of temperatures on infection and sporulation of *B. dothidea* on pistachio fruits and leaves. Inoculated fruits were not infected at 15, 36, or 39 C after 10 days of incubation (Figs. 4,5), but the percentage of fruit infection was more than 45% at 27–33 C and more than 95% after 5 and 10 days of incubation, respectively, in plastic containers with greater than 95% RH (Fig. 4). Pycnidia were produced at 21–33 C. The optimum temperature for the latter was 30 C. At 21 C, several lesions developed on fruit but remained small after 10 days, whereas at 24–33 C lesions coalesced and resulted in fruit blight (Fig. 5).

Two days after inoculation, initial symptoms were observed as small tan-to-black spots or small watersoaked lesions on leaves incubated at 24–33 C. After 5 days, symptoms of infection were evident on leaves incubated at 15 and 21 C. The optimum temperatures for disease development on leaves were 24–30 C, as determined by the size of the infected leaf area (Fig. 6). Pycnidia were not evident at any temperature after 5 days but were present on leaves incubated at 27–30 C after 10 days, on those at 21–24 C after 15 days, and a few developed at 15 C after 20 days. Although leaf infection occurred at 33 C, pycnidia were not formed after 20 days. The majority of the pycnidia developed on leaves incubated at 27 or 30 C (Fig. 6). Stems of leaves were infected also, particularly those incubated at 27–30 C, on which an abundance of pycnidia developed. Pycnidial density was lowest in the interveinal leaf areas.

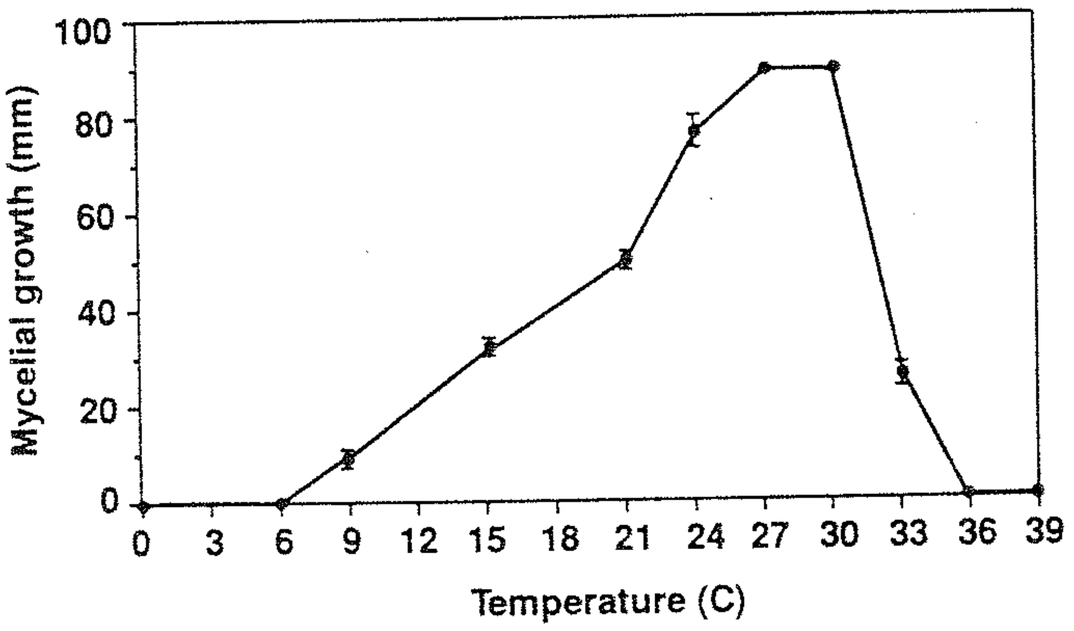

Fig. 3. Effect of temperature on mycelial growth of *Botryosphaeria dothidea* on acidified potato-dextrose agar after 6 days (LSD = 3.1 mm). Results represent the mean from two repetitions of the experiment.

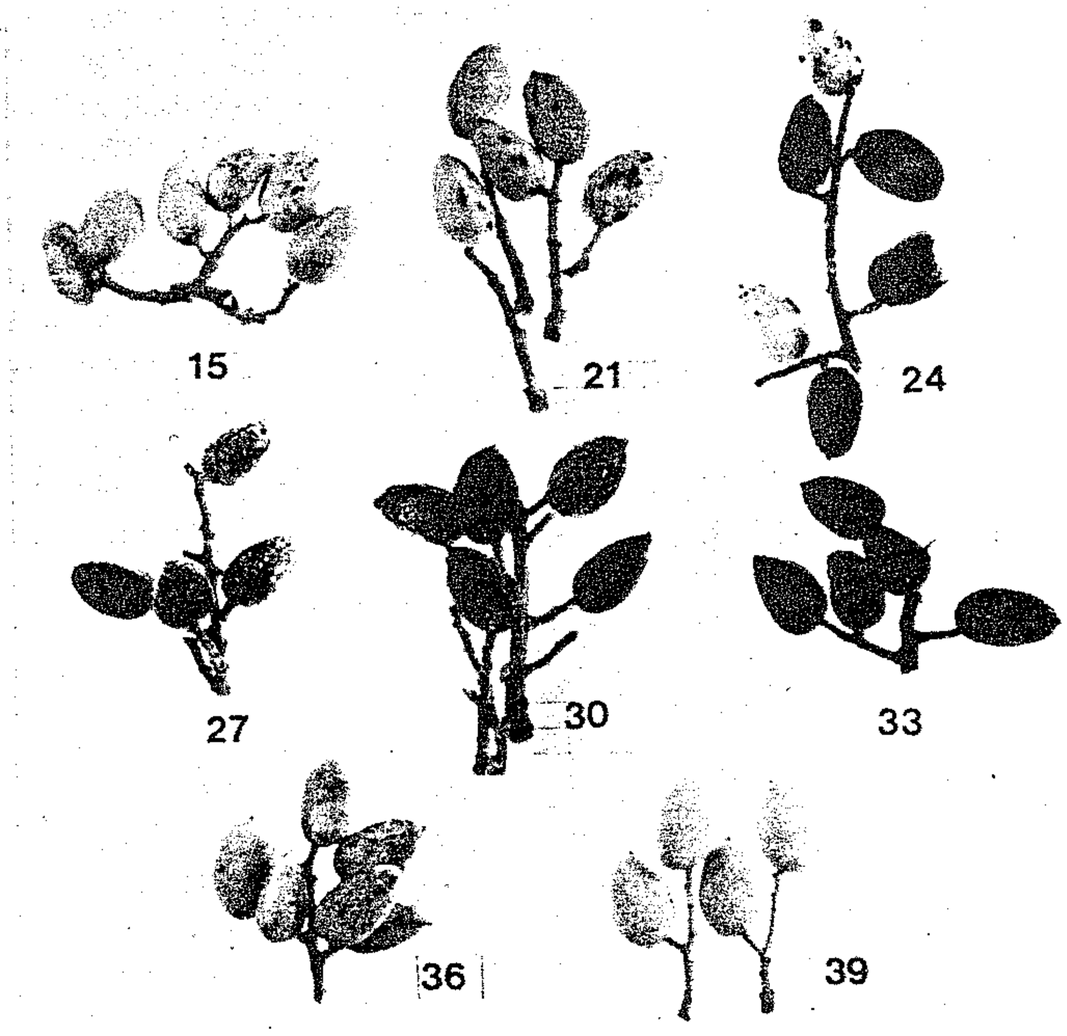

Fig. 5. Infection of pistachio (cv. Kerman) fruit after inoculation with a suspension of 10^5 spores per milliliter of *Botryosphaeria dothidea* (isolate Bd-5F) and incubation at different temperatures for (C) 10 days.

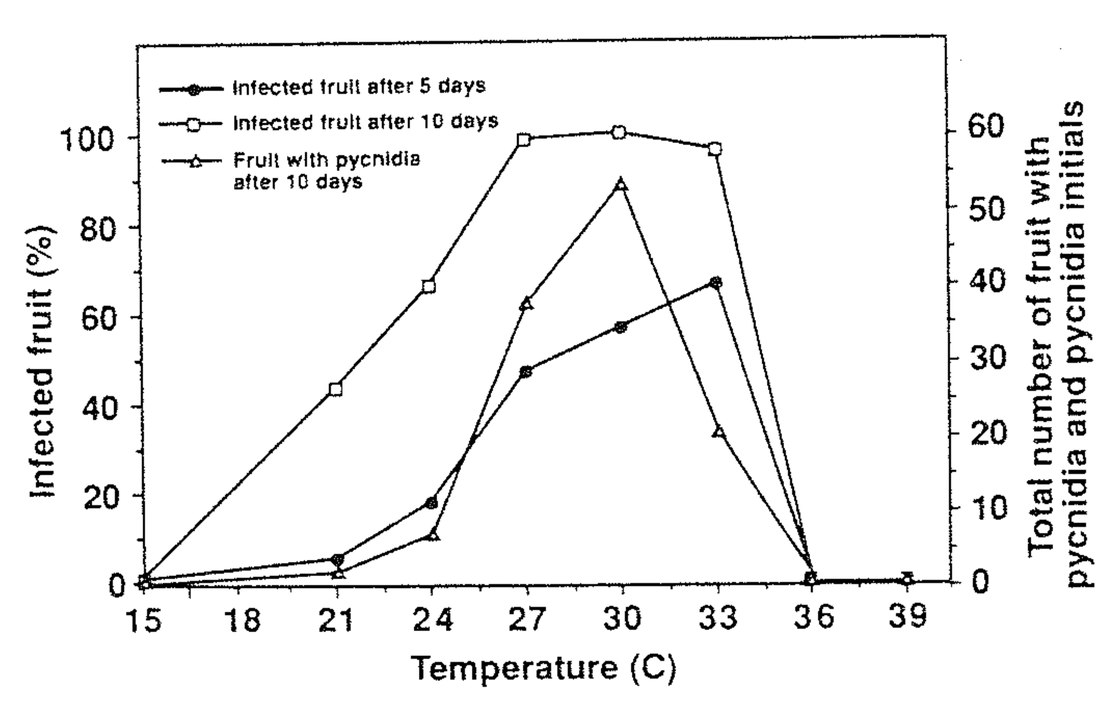

Fig. 4. Effect of temperature on infection of pistachio (cv. Kerman) fruit and pycnidia development after inoculation with *Botryosphaeria dothidea*. For percentage of infected fruits after 5 days, LSD = 17.2%; after 10 days, LSD = 10.1%; and fruits with pycnidia and pycnidia initials after 10 days, LSD = 1.7.

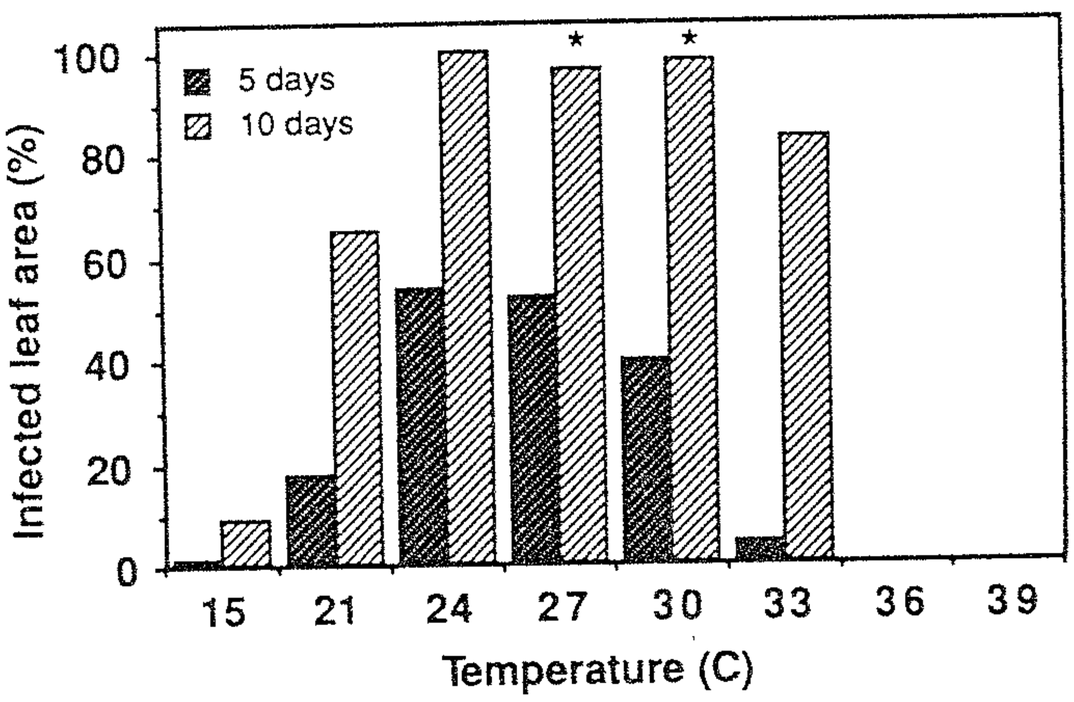

Fig. 6. Effect of temperature on infection of pistachio (cv. Kerman) leaves by *Botryosphaeria dothidea* after 5 (LSD = 21.1%) and 10 days (LSD = 14.3%) of incubation. The asterisks indicate development of pycnidia on leaves and stems after 10 days.

Latent periods of naturally infected pistachio fruit for pycnidia development at different temperatures. Temperature significantly affected the latent periods for pycnidia development ($P < 0.001$); significantly more pycnidia were formed on pistachio nuts at 33 than at 27 C after 4, 6, or 10 days (Fig. 7). After 4 days of incubation at 33 C, 65% of the nuts had pycnidia with oozing pycnidiospores. After 6 days, significantly more nuts developed pycnidia at 27 C (at which 70% of the nuts had pycnidia after 10 days) than at 6–20 C. However, at 10 C it took 10 days for 20% of the infected fruits to produce pycnidia, and at 6 C no pycnidia were formed even after 10 days of incubation (Fig. 7). The relationship between the incidence of naturally infected pistachio fruit that developed pycnidia and the temperature at which fruits were incubated for 10 days was best described (confirmed by the least-squares test) by the equation

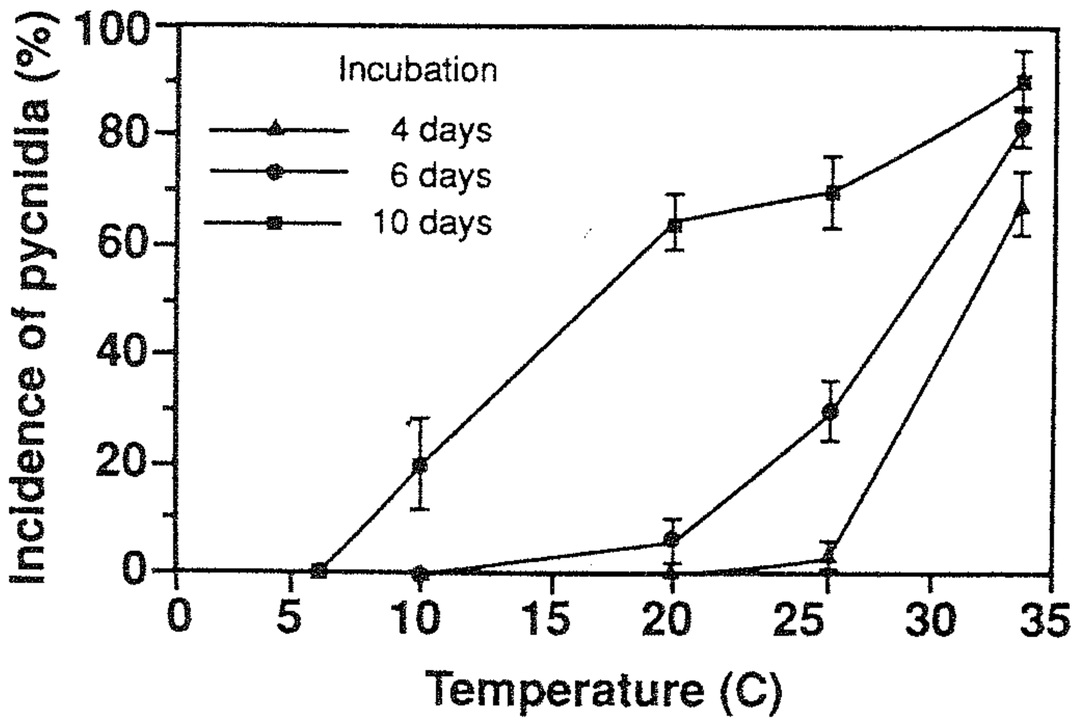

Fig. 7. Effect of temperature (6–33 C) on development of pycnidia on pistachio (cv. Kerman) fruits naturally infected by *Botryosphaeria dothidea*. Vertical lines represent standard errors.

$$Y = -33.22 + 6.09T - 0.07T^2 \quad (2)$$

in which Y = incidence of fruits with pycnidia, and T = incubation temperature; the coefficient of determination (R^2) was 0.88. The equation was significant at $P < 0.001$.

Effects of continuous wetness on infection of pistachio by *B. dothidea*. The incidence of leaves infected by *B. dothidea* increased with increasing wetness duration (Fig. 8). At 0–3 h of wetness duration, leaf symptoms were not observed. Incidence of infection increased to only 8 and 12% of the leaves after 6 and 9 h, respectively. However, 36–66% and 97–100% of the leaves were infected when they were kept wet for 12–24 h and 48–72 h, respectively (Fig. 8). The incidence of infected leaves was best described by the equation

$$Y = -27.610 + 6.051X - 0.104X^2 + 0.005X^3 \quad (3)$$

in which Y = percentage of infected leaves, and X = wetness duration; $R^2 = 0.99$. The equation was significant at $P < 0.01$.

Disease severity on leaves also increased with wetness durations from 18 to 72 h (Table 1). Longer wetness periods resulted in correspondingly higher percentages of leaves with multiple lesions per leaf (Table 1). With wetness durations of 6–12 h, all infected leaves had one to five lesions per leaf. With wetness durations of 18–24 h, more than 50% of the leaves had one to 10 lesions, and 3–8% had more than 11 lesions per leaf. However, approximately twice as many leaves had 11–15 or more than 16 lesions per leaf after 72 h of wetness than after 48 h (Table 1). In addition, the incidences of blighted leaves and petioles increased with increasing duration of wetness (Fig. 9) and were described by the equations

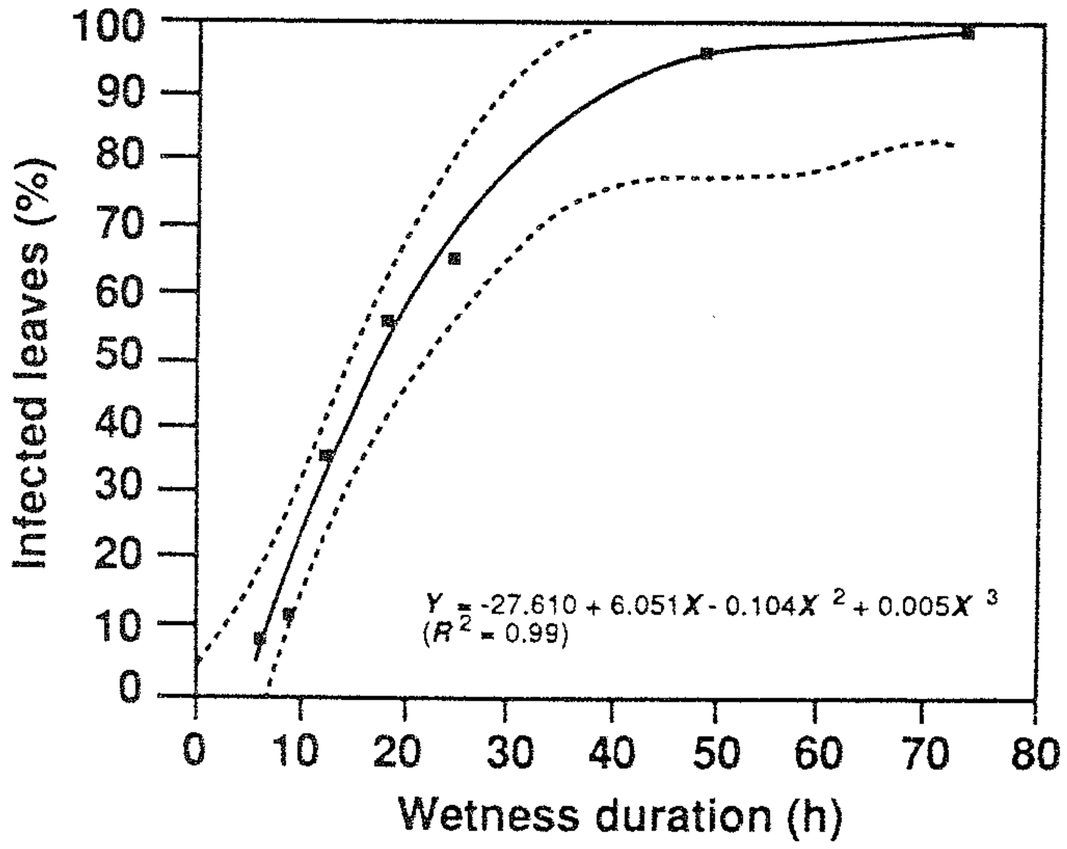

Fig. 8. Effect of continuous wetness periods on infection of leaves of pistachio (cv. Kerman) by *Botryosphaeria dothidea* in the field (R^2 is significant at $P < 0.01$). The dashed lines represent the upper and lower 95% confidence limits around the regression line.

$$Y = -8.52 + 0.56X \quad (4)$$

$$Y = 11.50 + 1.12X \quad (5)$$

in which Y = percentage of blighted leaves for Equation 4 and percentage of blighted petioles for Equation 5, and X = wetness duration; $R^2 = 0.88$ and 0.78 for Equations 4 and 5, respectively. Equations 4 and 5 were significant at $P < 0.05$ and $P < 0.01$, respectively. The least-squares method confirmed the linearity of the functions in Equations 4 and 5. *B. dothidea* was recovered from 80% of the black lesions developed on leaf blades and petioles.

TABLE 1. Effect of wetness duration on incidence and severity of infections of pistachio (cv. Kerman) leaves by *Botryosphaeria dothidea* at Wolkskill Experimental Orchards in Winters, California

Wetness duration (h)[v]	Leaves with lesions (%)[w,y]					Disease index[x,y]
	0	1–5	6–10	11–15	≥16	
18	44.0 ± 4.8[z]	51.0 ± 8.4	2.0 ± 1.1	0.0 ± 0.0	3.0 ± 1.8	0.63 ± 0.11
24	34.1 ± 7.3	48.1 ± 4.5	10.4 ± 4.2	3.7 ± 1.0	3.7 ± 1.9	1.03 ± 0.16
48	3.1 ± 2.5	40.6 ± 8.5	31.3 ± 5.9	12.5 ± 2.8	12.5 ± 7.1	1.88 ± 0.21
72	0.0 ± 0.0	21.7 ± 4.0	30.4 ± 5.9	21.8 ± 3.3	26.1 ± 7.5	2.48 ± 0.27

[v] Inoculations were made on 15 September 1987 and 1989 by spraying leaves on current growth shoots with 10^5 spores of *B. dothidea* per milliliter until run-off and covering the shoots with polyethylene and paper bags.
[w] All the leaves on each shoot were evaluated 20–25 days later. All infected leaves kept wet for 6–12 h had only one to five lesions per leaf.
[x] Disease index was based on a scale of 0–4, in which 0 = healthy (no lesions), 1 = 1–5 lesions per leaf, 2 = 6–10, 3 = 11–15, and 4 = ≥16 and was calculated by using Equation 1. Disease index rating for shoots sprayed with water (controls) ranged from 0.02 to 0.23 (significantly different from the inoculated treatments at $P < 0.01$).
[y] Results are expressed as an average of five replications for each wetness duration.
[z] Numbers (±) represent standard errors.

Measurable amounts of infected fruits (fruits with black lesions on the epicarp) occurred with 18 h of continuous wetness and increased significantly with wetness duration, for example, with 18–24 h, 8–21% of fruit clusters and 15–27% of fruits were blighted (2–5% of the fruit had mature pycnidia). In contrast, with 48–72 h, 40–100% of the fruit clusters and 61–89% of the fruits were blighted (11–18% of them had developed mature pycnidia). The incidences of blighted clusters, blighted fruits, and blighted fruits bearing mature pycnidia were increased with increasing duration of wetness and were best described by the following linear equations

$$Y = -22.8 + 1.60X \quad (6)$$

$$Y = -6.84 + 1.35X \quad (7)$$

$$Y = -2.9 + 0.29X \quad (8)$$

in which Y = percentage of blighted clusters for Equation 6, percentage of blighted fruits for Equation 7, and percentage of blighted fruits bearing mature pycnidia for Equation 8, and X = wetness duration; $R^2 = 0.94$, 0.99, and 0.99, respectively. Equation 6 was significant at $P < 0.05$, and Equations 7 and 8 at $P < 0.01$.

Effects of interrupted wetness periods on disease incidence and severity. Percentage of infected leaves increased with additional 12-h wetness periods and was best described (confirmed by the least-squares test) by a linear regression equation (Fig. 10). Severity of disease increased with three or four additional 12-h wetness periods (Table 2). For instance, with one or two 12-h wetness periods, only up to 14% of the infected leaves had more than 10 lesions per leaf, but with three or four 12-h wetness periods, 30–65% of the infected leaves had more than 10 lesions per leaf, respectively (Table 2). Disease severity index increased exponentially with additional 12-h wetness periods (Fig. 10). Both regressions were significant ($P < 0.01$).

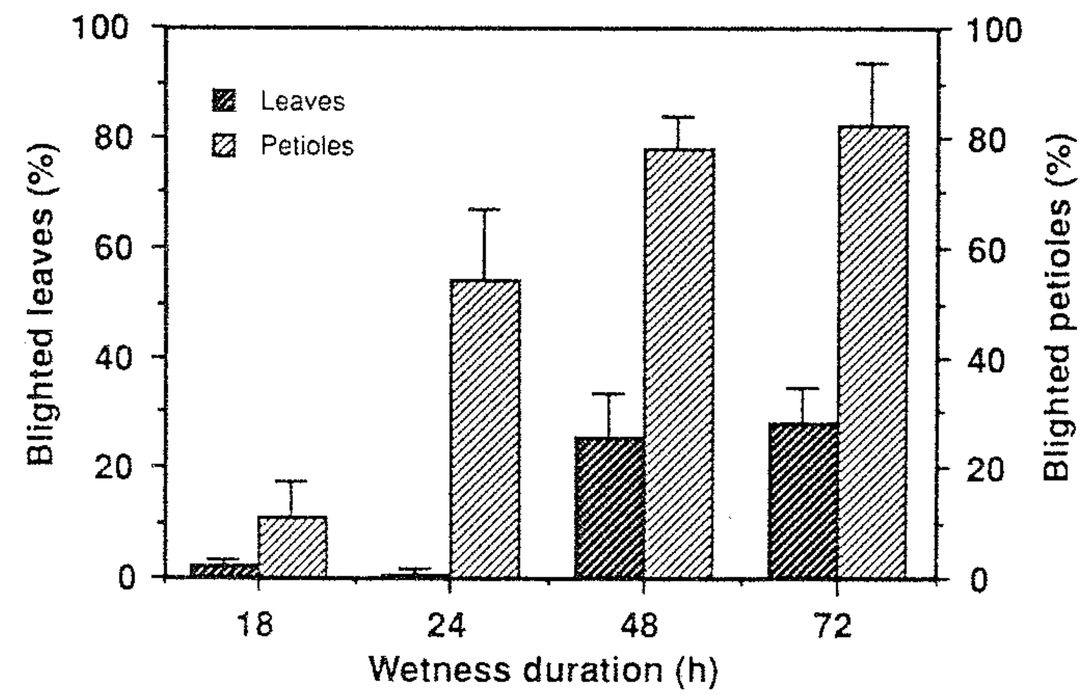

Fig. 9. Effect of continuous wetness period from 18 to 72 h on the incidence of blighted pistachio (cv. Kerman) leaves and petioles, after inoculation with a suspension of 10^5 spores per milliliter of *Botryosphaeria dothidea* in the field. Vertical lines represent standard errors.

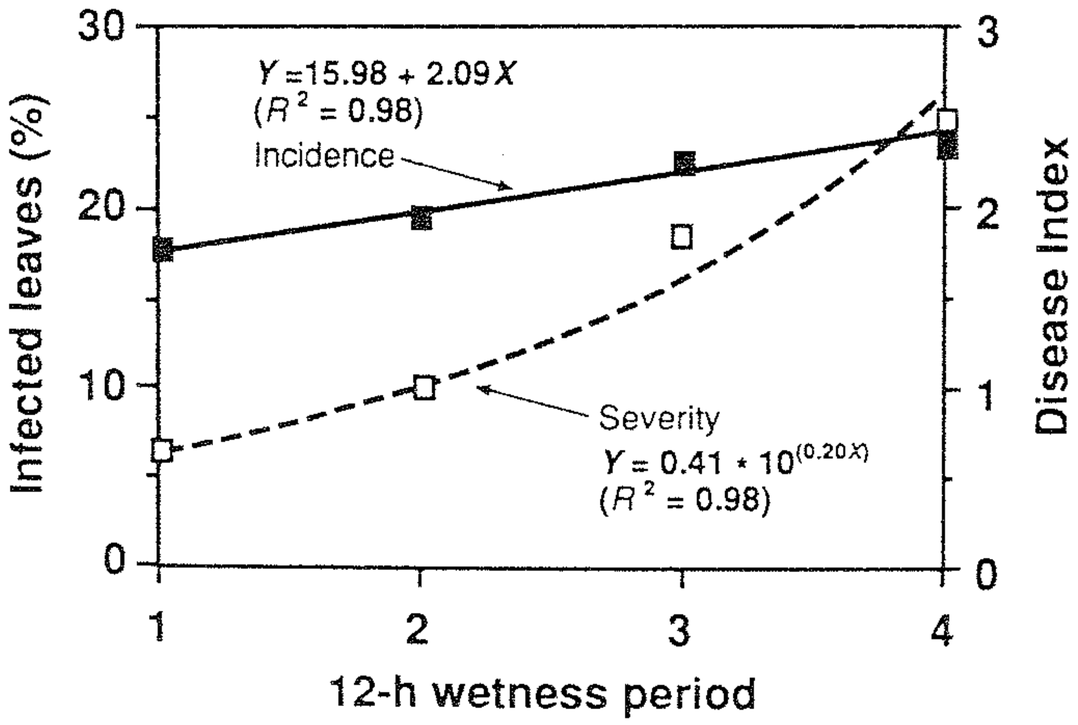

Fig. 10. Effect of one to four interrupted 12-h wetness periods on incidence (■) and severity (□) of infected pistachio (cv. Kerman) leaves inoculated with a suspension of 10^5 spores per milliliter of *Botryosphaeria dothidea*. R^2 for incidence and severity are significant at $P < 0.01$.

Control of disease by reducing duration of irrigation. In 1989, in the Butte County experimental plot, trees in blocks irrigated for 24 h per irrigation showed 31% infected fruits, whereas those in blocks irrigated for 12 h had only 20% (results not shown). The differences were significant at $P = 0.10$. In 1990, when disease levels in general were higher, about 60% of the fruits on trees in blocks irrigated for 24 h were infected compared with 48% of fruits collected from trees in blocks irrigated for 12 h (Table 3). This difference was significant at $P = 0.08$. There were also trends toward reduced percentages of fruits with pycnidia ($P = 0.10$) and infected rachises ($P = 0.15$) with the 12-h duration of irrigation (Table 3). Incidences of blighted rachises, infected leaves, and disease severity index of leaves were not affected by the short duration of irrigation. Blighted rachises had their entire tissue killed, whereas in infected rachises only lateral branches or the upper part of main axis was killed.

In 1991, however, reduction of irrigation duration to 12 h significantly reduced all factors, percentage of infected fruits ($P < 0.01$), fruits bearing pycnidia ($P < 0.05$), infected ($P < 0.0001$) and blighted rachises ($P < 0.001$), and infected leaves ($P < 0.001$). In addition, fewer lesions (lower disease index [$P < 0.001$]) developed on leaves collected from trees irrigated for 12 h than from trees irrigated for 24 h, which had two times greater severity index than trees irrigated for the same duration in 1990 (Table 3).

In the San Joaquin County orchard, the incidence of infected leaves (37%) was significantly ($P < 0.01$) lower in trees irrigated for 24 h than in those collected from trees irrigated for 48 h. In addition, leaf disease severity index was significantly ($P < 0.0001$) reduced in trees irrigated for 24 h than those irrigated for 48 h (Table 3).

DISCUSSION

Temperature and duration of wetness directly affect infection of pistachio by *B. dothidea*. Optimum temperatures for disease development (27–30 C) and sporulation (30 C) on pistachio fruits and leaves corresponded closely to those (27–30 C) for mycelial growth and pycnidiospore germination of *B. dothidea*, which explains why the disease is most severe during summer and early fall (12). Air temperatures of 27–33 C are common during summer and early fall in all of the pistachio-growing areas in California. Kohn and Hendrix (11) found that the temperatures at which lesion size on apples caused by *B. dothidea* increased most rapidly

TABLE 2. Effects of interrupted wetness periods on the severity of infections of pistachio (cv. Kerman) leaves by *Botryosphaeria dothidea* at Kearney Agricultural Center in Parlier, California

Wetness periods (h)[x]	Leaves with lesions (%)[y]			
	1–5	6–10	11–15	≥16
12	12.1 ± 3.2[z]	3.4 ± 4.0	1.2 ± 2.3	1.3 ± 1.8
12 + 12	14.1 ± 5.5	4.5 ± 3.4	0.2 ± 0.8	1.0 ± 3.1
12 + 12 + 12	9.2 ± 5.9	6.6 ± 2.9	3.9 ± 5.7	3.3 ± 6.6
12 + 12 + 12 + 12	5.5 ± 4.8	2.9 ± 2.6	5.5 ± 0.8	9.9 ± 4.0

[x] Inoculations were made on 26 October by spraying to run-off leaves on current growth shoots with 10^5 spores per milliliter; and shoots were covered with a polyethylene bag inside and a paper bag outside and were uncovered the next morning; they were left uncovered for 12 h and wetted with water for subsequent wetness periods.

[y] All (12–36) leaves were evaluated for disease 15 days after the last wetness period.

[z] Numbers (±) represent standard errors.

were in the range of optimum growth of the pathogen in culture. This study also shows that *B. dothidea* infected unwounded pistachio fruits sprayed with a spore suspension of the fungus. In another study (12), it was shown that spores penetrated through lenticels and infected pistachio fruit. In contrast, in other kinds of fruits, such as apples, wounding is necessary for fruit infection (2). This difference could be explained by the different structures of the surface of these fruits; apples are known for their thick epicuticular layer of wax (24).

Under favorable wetness conditions, temperatures after fruit inoculation significantly influenced the levels and intensity of infection and the development of pycnidia. The optimum temperature for pycnidial initials and mature pycnidia was 30 C. At 27 C, for instance, 45% of the fruits were infected after 5 days, whereas it took 10 days to reach the same rates of infection at 21 C (Fig. 4). Temperature also influenced the number of lesions developed on apple leaves by *B. obtusa* (6). Bulger et al (3) showed that with increasing duration of wetness (8–32 h) at 15–25 C, infection of flowers and fruits of strawberry by *Botrytis cinerea* increased. They also found that the disease was significantly less above 25 C and below 15 C. Similarly, *B. dothidea* caused lower levels of disease (incidence and reproduction) below 27 C or above 33 C. The low infection rates above 33 C observed in this study can be explained by the inability of conidia to grow and develop colonies at those temperatures and by the reduced growth of fungus at lower temperatures (Fig. 3).

In commercial pistachio orchards, pycnidia on infected fruits and leaves infected by *B. dothidea* were first noticed in August (when daily maximum, minimum, and average temperatures ranged from 27 to 39, 10 to 18, and 19 to 27 C, respectively, recorded about 3 km south of the pistachio orchard in Butte County). From August through early September, a combination of such inoculum increases (pycnidia development), and infection-conducive temperatures and wetness conditions contributed to disease outbreaks in pistachio orchards in northern California (18).

Latent period for development of pycnidia in naturally infected fruit shortened as temperature increased. For instance, 90, 70, and 20% of the infected fruits developed pycnidia at 33, 20, and 10 C, respectively, after 10 days of incubation. Therefore, provided that adequate moisture is available, temperatures of 27–33 C favor germination of pycnidiospores, infection, incubation, and latent periods and reproduction of *B. dothidea*; this explains why the disease increased to epidemic levels during the late summer.

In general, incidence and severity of disease increased with increasing wetness duration up to 72 h. Although pycnidiospores of *B. dothidea* can germinate within 1.5–2.0 h after the initiation of a wetness period at 23–30 C, the majority of them did not penetrate until after 12 h (12). After wetness periods of 9–12 h, none of the petioles was infected. At wetness durations of longer than 12 h, significantly greater percentages of fruits were infected or blighted, larger numbers of lesions per leaf developed, and infection and blight of petioles and leaves occurred. Under such conditions, the disease can cause significant fruit losses due to fruit blight and tree defoliation due to leaf blight. Severe petiole and rachis infections that lead to leaf and fruit blights were commonly observed yearly in an orchard in Tehama County where sprinklers with a high (23°) trajectory angle continuously wetted the lower half of the tree canopy for at least 24–32 h per irrigation (T. J. Michailides, *unpublished*). Increased disease incidence and severity with increased duration of wetness have been reported for several other leaf and fruit diseases (6,23).

Continuous wetness duration of longer than 12 h favored incidence and severity of disease development (Figs. 8,9; Table 1). Similarly, Arauz and Sutton (1) showed that wetness duration affected the density of lesions caused by *B. obtusa* on apple leaves. For the majority of germinated spores of *B. dothidea* to penetrate and subsequently infect, longer than 12 h of continuous wetness duration is required. Multiple penetrations through stomata were observed 24–48 h after inoculation and incubation of pistachio leaves in a moist environment (12), a fact that can explain the high levels of disease (Fig. 8) and the multiple (>16) lesions developed per leaf (Table 1).

That incidence and severity of disease increased with recurring 12-h wetness periods can be explained by the behavior of *B. dothidea* spores under alternating wet-dry treatments. In the course of intermittent rainfall, conidia that germinated during an initial short rain or an irrigation-induced wetness period can survive at least a 12-h dry period. Moreover, a number of spores (≃50%) develop thick walls, become dark and septate, and remain in this condition during the initial wet period and a subsequent dry cycle postpones their germination for a following favorable wet cycle (rain or sprinkler irrigation) (T. J. Michailides, *unpublished*).

Information obtained from the experiments on wetness duration can be utilized by growers to manage disease by the manipulation of irrigation practices. For example, in 1989 and 1990 in the orchard in Butte County where duration of irrigation was reduced to 12 h per irrigation from 12 + 12 h (in two consecutive days), Botryosphaeria panicle and shoot blight was reduced from 31 and 60% to 20 and 48% ($P \leq 0.10$), respectively, and in 1991 from 62 to 19% ($P < 0.01$). The irrigation records for this commercial orchard in Butte County indicate how critical periods of wetness duration are in causing disease epidemics. In 1986, when duration of irrigations ranged from 22 to 46 h, 76% of the pistachio fruits were infected (18). In 1988, when the grower applied three irrigations of 44 h each before harvest, 99% of the fruit was lost because of infection by *B. dothidea*, the highest loss recorded in this orchard (18).

Similarly, on the basis of the experiments in San Joaquin County orchard, control of the disease is also possible in orchards

TABLE 3. Effect of reducing duration of sprinkler irrigation on Botryosphaeria panicle and shoot blight of pistachio caused by *Botryosphaeria dothidea* in orchards in Butte County (1990 and 1991) and San Joaquin County (1991), California

Year	Orchard	Duration of irrigation (h)	Infected fruit (%)[v]	Fruit with pycnidia (%)[v]	Infected rachises (%)[w]	Blighted rachises (%)[w]	Infected leaves (%)[x]	Leaf disease index[y]
1990	Butte Co.	24	59.7 a	17.3 a	64.4 a	23.1 a	37.5 a	0.86 a
		12	47.7 a	12.8 a	53.9 a	18.0 a	38.1 a	0.87 a
LSD ($P < 0.05$)			13.6	5.8	14.4	12.2	11.5	0.55
1991	Butte Co.	24	62.0 a	17.4 a	68.9 a	26.6 a	74.0 a	1.73 a
		12	19.2 b	4.9 b	16.8 b	1.0 b	30.5 b	0.47 b
LSD ($P < 0.05$)			23.0	11.1	9.8	8.7	15.1	0.68
1991	San Joaquin Co.	48	...[z]	...	...	...	96.8 a	2.94 a
		24	...	...	...	...	37.0 b	0.60 b
LSD ($P \leq 0.05$)							21.3	0.50

[v] Averages of six 200-fruit replications.
[w] Averages of six 30–50 rachises.
[x] Fifty leaves per tree were evaluated.
[y] Disease index was calculated by using Equation 1 and was based on five disease severity categories: 0 = healthy (no lesions), 1 = 1–5 lesions per leaf, 2 = 6–10, 3 = 11–15, and 4 = ≥16.
[z] Not determined because of prescheduled harvest by the grower.

with permanent sprinkler irrigation systems. Disease incidence and severity were even greater in this orchard than in the orchard in Butte County (Table 3), probably because durations of irrigations were longer (48 h). Consequently, the reduction of incidence and severity of disease by reducing duration of irrigation to 24 h was very drastic. Reduction of duration of irrigation did not appear to stress the trees. Because water stress has been reported to predispose trees to diseases caused by *B. dothidea* (19,22), pistachio growers should consider any adverse effects from tree water stress when they reduce duration of irrigation for controlling the disease and avoid imposing water stress in pistachio.

Because rains usually do not occur in late spring, summer, and early fall in California, the dependence on free water for infection by *B. dothidea* makes control of this disease in the field possible by adjusting the duration of sprinkler irrigation to minimize wetness duration. Overnight irrigation increases the chances of leaf surfaces remaining wet for extended periods, thus favoring an increase in infection, although cooler night temperatures that are unfavorable for infection could compensate this disease increase. However, we have shown that reducing duration of irrigation from 24 to 12 h and irrigating only during daytime reduce the disease significantly. Reducing duration of sprinkler irrigation reduces wetness duration and inoculum production and probably inoculum spread.

Results obtained from this study along with additional studies on the interactions between temperature and wetness duration can be used for developing a predictive model for this disease that can be affected by environmental conditions and methods of orchard irrigation. For instance, orchards irrigated by high-angle sprinklers for 48 h per irrigation are at high risk of being damaged by the disease, a situation commonly found in pistachio orchards in Butte, Tehama, and Glenn Counties in northern California. In contrast, in orchards irrigated by drip or microjet sprinklers (which do not wet the tree canopy or the entire orchard floor), the disease does not pose a major threat. Therefore, although *B. dothidea* has been isolated from pistachio trees throughout California orchards (12), to date it has caused major crop losses only in northern California orchards where sprinkler irrigations are common and last 22–48 h.

Because temperature in the orchard cannot be controlled to any great extent, it is easier to base recommendations for disease control on a practice over which we have some degree of control (i.e., duration of irrigation). This approach to controlling the disease is timely because, due to the recent 5-yr drought in California, sustained or controlled deficit irrigations (irrigation not fulfilling the 100% evapotranspiration requirements) have been studied and are presently recommended for pistachio in California (7–9).

LITERATURE CITED

1. Arauz, L. F., and Sutton, T. B. 1990. Effect of interrupted wetness periods on spore germination and apple infection by *Botryosphaeria obtusa*. Phytopathology 80:1218-1220.
2. Brown, E. A., II, and Britton, K. O. 1985. Botryosphaeria diseases of apple and peach in the southeastern United States. Plant Dis. 70:480-484.
3. Bulger, M. A., Ellis, M. A., and Madden, L. V. 1987. Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence of fruit originating from infected flowers. Phytopathology 77:1225-1230.
4. California Pistachio Commission. 1990. California pistachio 1988 acreage-bearing/non-bearing production and yield. Page 47 in: California Pistachio Industry, Annu. Rep. Crop Year 1989-1990. California Pistachio Commission, Fresno. 136 pp.
5. DuPont. 1988. Benlate 50 DF fungicide control of shoot blight on pistachios in the State of California. Supplemental labeling for distribution and use only within California. EPA Reg. No. 352-447. Agric. Bull. E.I. DuPont de Nemours & Co. Agric. Prod. Dep. 1 p.
6. Foster, H. H. 1937. Studies on the pathogenicity of *Physalospora obtusa*. Phytopathology 27:803-823.
7. Goldhamer, D. A., Beede, R., and Dunai, J. 1990. Controlled deficit irrigation with mature pistachio trees: First year results. Pages 73-76 in: California Pistachio Industry, Annu. Rep. Crop Year 1989-1990. California Pistachio Commission, Fresno. 136 pp.
8. Goldhamer, D. A., Beede, R., and Fusi, S. 1991. Effects of deficit irrigation timing on pistachio tree productivity. Pages 84-89 in: California Pistachio Industry, Annu. Rep. Crop Year 1990-1991. California Pistachio Commission, Fresno. 136 pp.
9. Goldhamer, D. A., Beede, R., Fusi, S., and Van Brocklin, F. 1992. Controlled deficit irrigation on mature pistachio trees: Third year results. Pages 74-79 in: California Pistachio Industry, Annu. Rep. Crop year 1991–1992. California Pistachio Commission, Fresno. 172 pp.
10. Kliejunas, J. T. 1976. Bleeding canker of Norfolk island pine in Hawaii. Plant Dis. Rep. 60:84-87.
11. Kohn, F. C., Jr., and Hendrix, F. F. 1982. Temperature, free moisture, and inoculum concentration on the incidence and development of white rot of apple. Phytopathology 72:313-316.
12. Michailides, T. J. 1991. Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on pistachio. Phytopathology 81:566-573.
13. Michailides, T. J., and Morgan, D. P. 1992. Control of Botryosphaeria panicle and shoot blight of pistachio by reducing duration of irrigation. Pages 93-100 in: California Pistachio Industry, Annu. Rep. Crop Year 1991-1992. California Pistachio Commission, Fresno. 172 pp.
14. Michailides, T. J., Morgan, D. P., Grant, J. A., and Olson, W. H. 1990. Management of Botryosphaeria panicle and shoot blight in pistachio orchards through manipulation of irrigation practices. Pages 102-108 in: California Pistachio Industry, Annu. Rep. Crop Year 1989-1990. California Pistachio Commission, Fresno. 136 pp.
15. Michailides, T. J., Morgan, D. P., Grant, J. A., and Olson, W. H. 1991. Further attempts for management of Botryosphaeria panicle and shoot blight in pistachio orchards by altering irrigation practices. Pages 95-99 in: California Pistachio Industry, Annu. Rep. Crop Year 1990-1991. California Pistachio Commission, Fresno. 136 pp.
16. Michailides, T. J., and Ogawa, J. M. 1986. Sources of inoculum, epidemiology, and control of Botryosphaeria shoot and panicle blight of pistachio. Pages 87-91 in: California Pistachio Industry, Annu. Rep. Crop Year 1985-1986. California Pistachio Commission, Fresno. 129 pp.
17. Michailides, T. J., and Ogawa, J. M. 1988. Progress report on Botryosphaeria panicle and shoot blight of pistachio. Pages 46-52 in: California Pistachio Industry, Annu. Rep. Crop Year 1987-1988. California Pistachio Commission, Fresno. 91 pp.
18. Michailides, T. J., and Ogawa, J. M. 1989. Epidemiology, epidemics, and control of Botryosphaeria panicle and shoot blight of pistachio caused by *Botryosphaeria dothidea* in northern California. Pages 99-106 in: California Pistachio Industry, Annu. Rep. Crop Year 1988-1989. California Pistachio Commission, Fresno. 112 pp.
19. Pusey, P. L. 1989. Influence of water stress on susceptibility of nonwounded peach bark to *Botryosphaeria dothidea*. Plant Dis. 73:1000-1003.
20. Ramos, L. J., Lara, S. P., McMillan, R. T., Jr., and Narayanan, K. R. 1991. Tip dieback of mango (*Mangifera indica*) caused by *Botryosphaeria ribis*. Plant Dis. 75:315-318.
21. Rice, R. E., Uyemoto, J. K., Ogawa, J. M., and Pemberton, W. M. 1985. New findings on pistachio problems. Calif. Agric. 39(1):15-18.
22. Schoeneweiss, D. F. 1981. The role of environmental stress in diseases of woody plants. Plant Dis. 65:308-314.
23. Shaw, D. A., Adaskaveg, J. E., and Ogawa, J. M. 1990. Influence of wetness period and temperature on infection and development of shot-hole disease of almond caused by *Wilsonomyces carpophilus*. Phytopathology 80:749-756.
24. Walter, T. E. 1967. Russeting and cracking in apples: A review of world literature. Pages 83-95 in: East Malling Res. Stn. Annu. Rep. 1966.
25. Witcher, W., and Clayton, C. N. 1963. Blueberry stem blight caused by *Botryosphaeria dothidea* (*B. ribis*). Phytopathology 53:705-712.
26. Worral, J. J., Correll, J. C., and McCain, A. H. 1986. Pathogenicity and teleomorph-anamorph connection of *Botryosphaeria dothidea* on *Sequoiadendron giganteum* and *Sequoia sempervirens*. Plant Dis. 70:757-759.

Environmental Influence on the Infection of Wheat by *Mycosphaerella graminicola*

A. M. Magboul, S. Geng, D. G. Gilchrist, and L. F. Jackson

First author: assistant professor, Department of Plant Protection, King Faisal University at Al-Hassa, Saudi Arabia; second and fourth authors: professor and agronomist, respectively, Department of Agronomy and Range Science, University of California, Davis (UCD), CA 95616-8515; and third author: professor, Department of Plant Pathology, UCD.
Accepted for publication on 17 August 1992.

ABSTRACT

Magboul, A. M., Geng, S., Gilchrist, D. G., and Jackson, L. F. 1992. Environmental influence on the infection of wheat by *Mycosphaerella graminicola*. Phytopathology 82:1407-1413.

Mycosphaerella graminicola (anamorph, *Septoria tritici*), the causal agent of Septoria tritici blotch on wheat, can cause a significant yield loss for most commercially grown wheat cultivars and in many wheat-growing areas. Environmental factors which affect infection and disease development such as light intensity, temperature, and moisture have been studied extensively. Yet, reports on specific effects of temperature and leaf wetness period on the infection process are limited. The objectives of this study were to quantify the effect of temperature and duration of leaf wetness and their interaction during the infection process on major disease components, to study the dynamic characteristics of these disease components, and to derive a mathematical expression for each disease component, as a function of infection temperature and leaf wetness period. Results showed that temperature is a critical determinant of wheat infection by *M. graminicola* and that the optimum temperature for the infection process does not depend on leaf wetness period. Furthermore, the initial conditions of temperature and leaf wetness period following inoculation not only affect the rate of disease development but also the asymptotic level of disease severity that ultimately may be achieved. Except for lesion length, the maximum rate of increase in all disease components responds linearly to changes in leaf wetness period, and quadratically to changes in temperature. The rate of lesion length expansion is mainly influenced by postinfection conditions. Of the four components of Septoria tritici blotch of wheat studied, lesion unit (number of average-sized lesions) per square centimeter is the most sensitive indicator of a successful infection.

Mycosphaerella graminicola (Fuckel) Schroeter (anamorph: *Septoria tritici*), the causal agent of Septoria tritici blotch on wheat, is a pathogen of worldwide importance (6,22) and causes a significant yield loss in many wheat-growing areas (6,7,16,18). Studies show that certain weather patterns such as wet weather and moderate temperature favor disease development (4,10,13, 18,20). Moisture in the form of rain or dew is required for successful infection (2,8,14) and plays an essential role in all stages of the infection cycle involving the anamorph (5,8,11,13–15). In the laboratory, conidial germination occurs on moist leaves within 12 h and penetration occurs only after 24 h (14). Royle et al (18) and Holmes and Colhoun (15) also reported that 12 h of leaf wetness is sufficient to allow infection to take place while Renfro and Young (17) found that 15 h of leaf wetness is minimal for infection. Hess and Shaner (12) and Shipton et al (21) reported that disease severity increases as the postinoculation moist period increases.

Previous investigators recognized the close association between moisture and infection and subsequent pathogen development in the host plant. Research into the specific effect of temperature and its interaction with moisture on the infection process, however, has been limited (15,17,23). Therefore, the objectives of this study were to quantify the effect of temperature and duration of leaf wetness and their interaction during the infection process on several disease components, study the dynamic characteristics of these disease components, and develop a mathematical relationship between each disease component and the environmental factors of leaf wetness period and infection temperature.

MATERIALS AND METHODS

Plant material. Spring wheat cultivar Anza, susceptible to *M. graminicola*, was used in this study. Anza was chosen because preliminary studies showed that the leaves of this cultivar could endure long periods of wetness without becoming chlorotic or senescing prematurely under the conditions employed herein. Twenty 15-cm-diameter pots, each containing four plants, were used for each temperature experiment. Each experiment was repeated three times. The plants were grown under greenhouse conditions and were moved to growth chambers one week before inoculation.

Inoculum preparation. *M. graminicola* isolate DRT, a single pycnidiospore isolate obtained from field-infected Anza wheat near Davis, CA, was used in all experiments. Preliminary studies with isolate DRT under controlled environment conditions confirmed that it was pathogenic on a range of cultivars including Anza. Inoculum was increased on a solid medium containing 9 g of yeast extract, 9 g of malt extract, 9 g of sucrose, and 12 g of agar in 900 ml of distilled water. The cultures, incubated 6–8 days in a 12-h photoperiod at 20 C, grew as the anamorph (*S. tritici*) and displayed yeastlike growth by formation of blastospores without development of pycnidia. Blastospores were washed from the agar surface and collected in a glass of distilled water. The inoculum concentration was adjusted with a hemacytometer to 10^6 spores per milliliter of water containing 1 ml of Tween 20 per liter. This inoculum concentration gave reproducible infection level based on preliminary studies of the effect of inoculum concentration on disease severity.

Infection chamber. A misting system was developed to provide a fine layer of moisture on the leaf surface required for infection. The major parts of this system included a 1/4J air atomizing nozzle consisting of fluid cap no. 2050 and air cap no. 73328 (Spraying Systems Co., Wheaton, IL), master timer, day timer, night timer, pressure regulator, liquid strainer, air filter, and two solenoid valves. The air atomizing nozzle was placed beneath the plants within the growth chamber to avoid direct spray on the plants, and the water was supplied to the nozzle under pressure. Air and deionized water were internally mixed to produce a completely atomized flat spray pattern. The spray nozzle was placed in such a way that the spray was aimed in the same direction as the airflow so that the smallest spray droplets would be carried by the air and deposited on the leaf surfaces. This technique maintained a uniform distribution of minute droplets on the leaf surfaces without runoff throughout the infection period.

Inoculation. Plants between growth stages 40 (flag leaves