Factors Affecting the Occurrence and Severity of Blackmold of Ripe Tomato Fruit Caused by Alternaria alternata

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ABSTRACT

Free moisture as dew deposition was essential for rapid germination of conidia in the absence of rainfall. Dew was deposited at ambient temperatures at or below 15 C, temperatures that were suboptimal for rapid germination of conidia. However, water soluble nutrients; e.g., glucose and fructose, on the fruit surface dissolved in the dew, stimulated germination of conidia at 6-15 C. Infection and symptom development were enhanced by prior exposure of fruit to ultraviolet (UV) and infrared radiation under field conditions, but they were not required for disease development under greenhouse conditions. Inoculation of ripe fruits frequently resulted in large, sunken lesions whereas inoculations of green fruit resulted in quiescent lesions that failed to enlarge after the fruit ripened. Sporulation was optimum at 27 C and was inhibited below 15 C and above 33 C. Extended periods of temperature at 15 C or below reduced numbers of conidia trapped in late September and October. Conidia were liberated in a diurnal pattern under field conditions, and maximum release occurred from 1200 to 1800 hours.

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Additional key words: fruit exudates, dew, epidemiology.

Blackmold of ripe tomato (*Lycopersicon esculentum* Mill) fruit caused by *Alternaria alternata* (Fr.) Keissl. (= *A. tenuis* Nees) (28), frequently causes substantial losses in California's canning tomato crop. The disease is characterized by fruit lesions that range in size from quiescent small flecks, 2-3 mm in diameter, to large lesions involving one third or more of the fruit surface (13). *A. alternata* often sporulates profusely on the large lesions which usually extend into the carpel wall and sometimes into the locules. The disease is especially severe following unseasonally early rains in September or October (4). Sporadic disease development occurs in the absence of rain but lesions are formed only on the upper surface where the fruit is not protected from the sky by the leaf canopy. Such limited distribution of fruit lesions was reportedly due to increased inoculum deposition (33) and sunburning or sun exposure (4, 20) but field observations and data presented in this report indicate that neither is responsible.

Rotem and Reichert (23) reported the importance of dew in the development of early blight epidemics in the semiarid regions of Israel, but dew has not been shown to be important in the development of blackmold of tomato. A number of reports (3, 9, 10, 14) have shown that surface washings from host plants stimulated spore germination or infection by various pathogens, while others (6, 15, 19, 24, 30) indicated that the addition of sugars to spore suspensions increased infection of inoculated plants. If free moisture (i.e., dew) is essential for infection by *A. alternata* in the absence of rainfall, perhaps water soluble nutrients on the fruit surface stimulate spore germination, growth, and penetration.

The importance of small quiescent lesions in green tomato fruit that might subsequently develop into larger lesions in ripe fruit has been alluded to in the literature. Koepsell (13) reported that incipient infections by *A. tenuis* in greenhouse-grown mature green fruit resulted in rot of ripe fruit. McColloch and Worthington (16), reported that incipient infections of mature green tomatoes, caused by *A. tenuis*, usually remained quiescent unless the green fruit were predisposed by extended exposure to low temperatures (10 - 0 C). Allison (1) suggested the solanine content in green tomatoes partly accounted for the failure of *Colletotrichum phomoides* to colonize green fruit. Stavely and Slana (31) observed that *A. alternata* invaded tobacco leaves, but production of a cicatrix by host cells around the infection in immature leaves, permanently stopped the fungus from advancing. Incipient lesions in tomato caused by *A. alternata* fail to expand but no explanation has been provided.

Studies on liberation and dissemination of Alternaria are numerous. Environmental factors, low humidity (11, 22, 27), daily maximum temperatures (8, 27), and wind velocities (11, 22, 25) have been associated with diurnal periodicity of conidial liberation. Seasonal variation has been noted in the catch of conidia with the highest numbers usually recorded when disease was most prevalent in the latter part of the growing season (21, 22, 25). Several workers have reported small catches of *A. alternata* during the winter months (8, 12, 25, 29) indicating a probable effect of low temperature on spore production. Although many investigations have been undertaken to study climatic factors affecting spore production, liberation and dispersal of *A. alternata*, only the study of Rotem (22) in Israel was conducted in a climate similar to that of the semiarid Central Valleys of California.

Thus, there are many unanswered questions regarding several aspects of the blackmold disease of tomato. Reported herein are the results of investigations of environmental factors affecting blackmold development and occurrence, the relative contribution of incipient infections on green fruit to subsequent development of rot of ripe fruit, and factors affecting inoculum potential of *A. alternata* in a semiarid climate.

MATERIALS AND METHODS.—Tomato plants for greenhouse studies were grown in UC Mix No. 2C (2) supplemented biweekly with ammonium nitrate. Cultivar Hardin Miniature was grown in 25.4-cm diameter plastic pots and the larger cultivar, VF145-B7879, in 19-liter cans. Cultivar VF145-B7879 was used in all field studies at Davis.

Isolates of *A. alternata* used in these studies were obtained from blackmold lesions on ripe tomato fruit. Fungus cultures were maintained on potato-dextrose agar (PDA) at room temperature. Single conidium isolates were subcultured from stock cultures at 4- to 6-month intervals. The fungus was grown on V-8 juice agar (17) for 5-7 days in cool-white fluorescent light (intensity .0338 cal cm^{-1} sec^{-2} 12-hour photoperiod) to produce conidia for germination and inoculation studies. Conidia were harvested by adding a small amount of water and gently rubbing the sporulating mycelial mat with a bent glass rod. The suspension of conidia was decanted into sterile distilled water containing 0.1% Tween-20, the number determined with a haemocytometer and the concentration adjusted to 10^4-10^5 conidia/ml. The suspension of conidia was sprayed onto the fruit on the vine. Following inoculation, the fruit were covered with polyethylene bags for 24-36 hours to maintain high humidity. Disease ratings were made 2-3 weeks after inoculated green fruit had ripened or, alternatively, 2-3 weeks after inoculation of ripe fruit.

Weather data were obtained from a spot climate recorder (26) for wind velocity and wind direction at heights of 1 m and 2.5 m; a hygrothermograph in a standard weather shelter for temperature and relative humidity (RH) at the height of 1.8 m in 1969 and 1970, and 18 cm above the soil surface in 1971 and 1972; a Taylor dew recorder (32) modified by the use of a ground plexiglass disk and attached to a 7-day clock for deposition and duration of dew. A Burkard 7-day volumetric spore trap (Burkard Manufacturing Co., Ltd., Rickmansworth, Herts, England) was maintained near the weather station in the experimental plots to monitor populations of airborne conidia. The trap orifice was 45 cm above the soil surface. The relative height of the trap orifice and the top of the plants varied due to plant growth, but was never closer than 10 cm.

RESULTS.—*Inoculum deposition on exposed fruit.*—Conidia of *A. alternata* were present on most fruit in the field whenever sampled. Mature green tomatoes exposed to the sky (exposed) and from under a canopy of leaves (unexposed) were harvested 28 September and 12 October 1971 (late season), sprayed with sterile distilled water and placed in covered plastic containers at room temperature. Upon ripening, fruit showed flecking characteristic of developing blackmold lesions. *A. alternata* was isolated from 70 of 96 exposed fruit and from 51 of 97 unexposed fruit. These results showed that inoculum was present on fruit shielded from the sky as well as on fruit exposed to the sky. Some of the exposed and unexposed fruit of both harvest dates were placed on a laboratory bench (approximately 27 C and 30% RH). When ripe, 87 of 105 exposed fruit showed flecking on the surface exposed to the sky; and *A. alternata* was recovered from 98 of 132 flecks from these fruit. None of the unexposed fruit on the laboratory bench developed blackmold symptoms. In addition, although the fruit harvested on 12 October had been subjected in the field to 67 hours $\leqslant$ 10 C, only flecking but no enlarged lesions of blackmold developed.

Effects of relative humidity and temperature on germination of conidia.—The effects of different RH at various temperatures on germination of conidia were tested in the laboratory. The temperatures, RH, and exposure times were chosen to coincide with actual weather conditions recorded over a 4-year period in a tomato field at Davis. Relative humidities greater than 90% seldom occurred when temperatures were above 20 C. Therefore, 5, 10, 15, and 20 C $\pm$ 0.1 C were chosen for testing, and were maintained by a differential respirometer water bath. Reagent grade saturated salt solutions (34) were used to maintain desired relative humidities ($\pm$ 1.0%) as follows: at 20 C: K_2SO_4 – 98%, KH_2PO_4 – 96.5%, and KNO_3 – 93.5%; at 15 C: K_2SO_4 – 99%, $Pb(NO_3)_2$ – 97% and KNO_3 – 95.5%; at 10 C: K_2SO_4 – 98.5%, $Pb(NO_3)_2$ – 98% and KNO_3 – 96%; and at 5 C: K_2SO_4 –98.5%, and KNO_3 – 96.5%. Glass-distilled water was used to maintain 100% RH at all temperatures, but it usually resulted in a light film of condensed water on the conidia. No condensation was observed at RH $<$ 100%.

Conidia from 4- to 7-day-old cultures of isolate $SSLC_3$ were harvested dry with a cyclone spore collector. Conidia were deposited in a Pasteur pipette sealed at the small end. The sealed end of the pipette was broken off and the conidia were blown out of the pipette and allowed to settle onto triangular-shaped slivers of glass coverslips in the bottom of a settling tower. These slivers were suspended over the saturated salt solutions or glass distilled water in the respirometer reaction vessels, as described by Dubin (7). At the end of the exposure period, the slivers were removed and placed on a drop of lactophenol cotton blue to fix the conidia and facilitate counting. Conidia with germ tubes were considered germinated. Percent germination was calculated on the first 100 conidia encountered in randomly chosen fields examined at $\times$ 160. Treatments were replicated four times and each experiment was repeated two to four times.

Over 6 hours of exposure to 98.5% RH at 20 C and more than 9 hours at 15 C were required for greater than 10% germination (Fig. 1). However, at 100% RH, over 30% germination occurred between 3 and 6 hours at 15 C. But, even after 12 hours of exposure to 100% RH, less than 5% germination occurred at 10 C and none at 5 C. These studies indicate that free moisture speeds germination at all temperatures. At or below 15 C in the absence of rain, dew formation is required if germination is to be completed.

Dew deposition on exposed fruit.—Under mid- to late-summer conditions at Davis, California, dew deposits occur only on the upper surfaces of fruit exposed to the sky. Dew deposits usually were not heavy enough to cause coalescence of droplets to the point of runoff; therefore, the undersides of exposed fruit remained dry. Data on duration of dew were collected from 15 July to 4 October 1972. The average dew period for days which had at least 1 hour dew deposit, was 6 hours, but there were 10-15 hours of dew on some days. Of the 79 days for which recordings were made, only 19 days had no dew deposits and 18 days had greater than 8 hours of dew. Throughout the season the average ambient temperature at dew point was 15 C. Once the dew point was reached, the

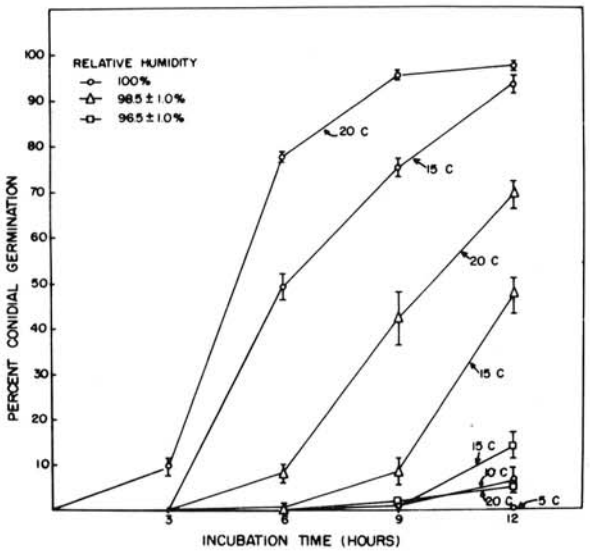

Fig. 1. Effect of temperature and relative humidity on the germination of conidia of *Alternaria alternata* on glass cover slips. Standard errors are indicated.

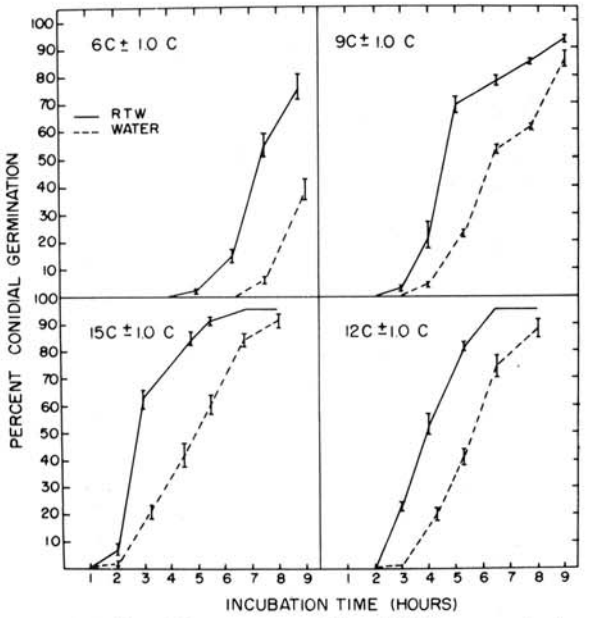

Fig. 2. Effect of ripe tomato washings (RTW) on germination of conidia of *Alternaria alternata* in water at four temperatures. Standard errors are indicated.

temperature usually continued to drop a few degrees.

Influence of fruit surface nutrients on germination of conidia.—Nutrients dissolved in dew deposits on ripe fruit surfaces were tested for their effect on the germination of conidia of *A. alternata*. All collections of fruit surface nutrients were made in the field during early morning hours from fruit laden with dew. The area of the fruit covered by a dew deposit was rinsed with approximately 3 ml of glass-distilled water applied with a Pasteur pipette. This liquid was termed ripe tomato fruit washings (RTW). Samples of RTW were centrifuged at 10,000 RPM for 15 minutes, sterilized by passage through a 0.22-μm, Millipore filter and lyophilized for use in analysis of constituents.

Conidia from 6- to 10-day-old cultures of isolate $SSLC_3$, harvested dry by scraping the sporulating mycelial mat with a wire loop avoiding nutrient contamination from the agar, were suspended in glass-distilled water, containing 0.1% Tween-20, and diluted to a concentration of 3-6 $\times$ 10^4 conidia/ml. One drop of the conidial suspension was placed on each of five spots in a plastic petri dish and one drop of test solution, dispensed from a 25 μl pipette, was immediately placed on top of each drop of spore suspension and the petri dishes were incubated at the desired temperature. The percent germination was determined by direct observation of the spore suspension droplets at hourly intervals, and after each observation the dishes were returned immediately to the incubators. All studies were repeated.

Ripe tomato washings of fruit collected from the field induced a stimulation of germination of conidia in water at low temperatures (Fig. 2). Conidia attained 50% germination in 7.5, 4.5, 4, and 2.75 hours at 6, 9, 12, and 15 C, respectively, in RTW-ammended suspensions. The stimulation of germination of conidia in RTW was most pronounced at 6 C, and less pronounced at the higher temperatures.

Lyophilized samples of RTW were separated into the neutral, cationic and anionic fractions to test their effect on germination of conidia at 12 C. To produce the neutral fraction, portions of RTW dissolved in 0.1 N HCl were passed through a column of Dowex-1 resin (Cl^- form). The Dowex-50 column was eluted with 4% NH_4OH to obtain the cationic fraction, and the Dowex-1 column with 0.1 N HCl for the anionic fraction. Each fraction was brought up to its original volume in glass-distilled water and all fractions were adjusted to pH 7.0 - 7.5 to approximate the original pH. Neither the anionic nor the cationic fractions stimulated germination of conidia. The neutral fraction stimulated germination of conidia to about the same extent as D-glucose, fructose, sucrose (each at 5, 25, and 50 μg/ml) and the original RTW compared to a water check in the same test.

Gas chromatography and mass spectrometry.—Gas chromatography and mass spectrometry were used to identify the carbohydrate constituents in RTW. Silylation for gas-liquid chromatography (GLC) was accomplished by adding 100 μl of Tri-Sil 'Z' (1.5 meq/ml N-trimethylsilylimidazole in pyridine, Pierce Chem. Co., Rockford, Ill.) (18) to lyophilized samples of RTW in microreaction vessels. Vials were heated to 65 C for 30 minutes and shaken. Trimethylsilyl (TMS) derivatives of reagent grade D-glucose and β-fructose were similarly prepared. Two microliter portions of the resulting solutions were injected directly into the GLC column.

The mass spectra of TMS derivatives were determined with a Finnigan 1015D gas-liquid chromatography-mass spectrometry (GLC-MS) instrument equipped with a glass column (2 mm $\times$ 1.5 m) packed with 3% OV-1 on 149-131 μm (100/120-mesh) Gas Chrom-Q. Operating conditions were: electron energy 70 e.v., source temperature 100 C, column temperature 140-210 C, a helium carrier gas flow rate of 40 ml/minute, mass range

TABLE 1. Blackmold disease severity on ripe tomato fruit as influenced by fruit maturity at time of infection by *Alternaria alternata* under greenhouse conditions

Cultivar and fruit maturity when inoculated	Total fruit (no.)		Fruit (% of total) with disease rating[a]			
	inoculated	diseased	1	2	3	4
			(% of total fruit)			
'Hardin Miniature'[b]						
A. Green	47	23	51	47	2	0
B. Green and ripe	47	41	13	34	38	15
C. Ripe	46	24	48	19	22	11
D. Uninoculated	50	7	86	10	4	0
'VF145-B7879'[c]						
A. Green	23	23	0	83	0	17
B. Green and ripe	24	24	0	21	29	50
C. Ripe	20	18	10	25	15	50
D. Uninoculated	27	3	89	11	0	0

[a]Disease rating scale: 1 = no symptoms; 2 = lesions small, shallow discrete flecks; 3 = lesion shallow, expanding laterally in epidermal and subepidermal tissue; 4 = lesion deep, extending into carpel wall or beyond, sometimes sunken.
[b]Results of analysis of variance: A vs. B and C; B vs. C; D vs. A and B and C significant at $P = 0.05$.
[c]Analysis of variance: A vs. B and C significant at $P = 0.01$; B vs. C not significant.

m/e 187 to 220, scan time 0.1 second, except that spectra were recorded at 1 second.

The three major peaks in the output from the total ion monitor of the GLC-MS instrument were identified as β-fructose, α-glucose, and β-glucose, by comparison of retention times and intensity of peaks between m/e 185 and 220 from the mass spectra with those of reagent grade D-glucose and β-fructose.

Importance of incipient infections under greenhouse and field conditions.—Isolation of *A. alternata* from incipient infections on green fruit collected from the field confirmed previous reports (13, 16) of the occurrence of incipient or quiescent infections by *A. alternata* on field-grown green fruit. Small groups of necrotic epidermal cells and necrotic trichomes on the fruit were located by examination with a stereoscopic dissecting microscope. Isolations from the affected cells yielded *A. alternata* in 35 of 75 small lesions of epidermal cells and 22 of 79 trichomes.

The small lesions of blackmold seldom enlarged on field-ripened fruit. Isolations from 3,362 arrested flecks and small lesions on field-ripened fruit over a 3-year period yielded *A. alternata*, 53%; *Stemphylium* spp., 18%; and various other fungi, 13%. No fungi were isolated from 16% of the lesions.

The maturity of the fruit at time of infection by *A. alternata* which resulted in the most severe symptom development at the ripe fruit stage was determined by inoculations in the greenhouse. Tomato (cultivars Hardin Miniature and VF145-B7879) fruit on the vine were inoculated by spraying with conidial suspensions of *A. alternata* (isolate $SSLC_3$). Each cultivar received four treatments as described in Table 1. The number of infected fruit and disease severity was recorded at harvest. Disease severity was categorized on the basis of a one-to-four rating system. Five flecks from each infected fruit were cultured on acidified PDA to determine the causal organism involved.

When green fruit at various stages of growth were inoculated, the lesions that developed on the ripened fruit were small and seldom enlarged. For example, infection of inoculated green fruit resulted in ratings of one or two. Inoculation of green fruit followed by reinoculation at the ripe stage resulted in lesions in all four categories with the majority rated as three or four. Inoculation of fruit only when ripe gave results similar to those obtained from the double inoculation.

These results indicate that incipient infections in green fruit were relatively unimportant under greenhouse conditions (21-27 C and 50-60% RH), but might not be applicable under field conditions. Therefore, studies were initiated to determine if blackmold severity was related to fruit maturity at the time of infection under field

TABLE 2. Blackmold disease severity on ripe tomato fruit as influenced by fruit maturity at time of infection by *Alternaria alternata* under field conditions

Planting date and treatment[a]	Total fruit bagged	Fruit (% of total) with disease rating[b]		
		1	2	3
1 May 1972				
1	72	0	31	69
2	26	31	69	0
3	126	1	61	38
15 May 1972				
1	50	0	44	56
2	75	20	68	12
3	43	2	91	7
30 May 1972				
1	44	0	48	52
2	99	12	78	10
3	66	1	79	20

[a]Treatments: 1 = green fruit of various sizes bagged until pink stage; 2 = green fruit of various sizes bagged and left covered until harvested; and 3 = green fruit exposed until pink, then bagged.
[b]Disease rating scale: 1 = no symptoms; 2 = mild symptoms (flecking); and 3 = severe symptoms (sunken lesions).

conditions. Fruit at various stages of maturity ranging from green to ripe, were covered with paper bags. Treatments were: (i) fruit covered during the green stage and uncovered during initial color change; (ii) fruit covered while green and remained covered until fully ripe; (iii) fruit covered at the pink stage and remained covered until harvest. All fruit covered during ripening were harvested approximately 2½ weeks after the fruit had ripened. All ripe fruit in the experiments were examined and rated from one to three for disease severity as follows: 1) no symptoms; 2) mild flecking symptoms; 3) severe sunken or much enlarged lesions. The experiment was repeated three times.

The results (Table 2) showed that exposure of fruit to inoculum in the ripe fruit stage resulted in more severe symptoms than exposure in the green stage. These findings agree with the greenhouse results and show that incipient infections of green fruit usually remain small and thus are relatively unimportant with respect to disease severity. Infections on ripe fruit produced most of the large sunken lesions. However, it should be noted that not all infections by *A. alternata* that occur after the fruit has ripened develop into severe lesions. It was noted, but not recorded quantitatively, that there was a marked increase in the number of quiescent lesions on fruit subjected to inoculum after ripening than on those fruit protected from the pink stage until ripe.

Influence of ultraviolet and infrared radiation on blackmold incidence under field conditions.—The effect of ultraviolet (UV) radiation on blackmold incidence was determined by suspending plastic film with different UV radiation transmission characteristics over tomato plants at the start of fruit set. Control plants were left uncovered. The plastics used and their respective percent transmittance of long-wave UV radiation were as follows: Mylar® polyester film, 150-μm (6-mil) (E.I. duPont de Nemours, Wilmington, Delaware), 12%; and Tedlar® PVF film, 50-μm (2-mil) (E.I. duPont, Delaware), 75%. Each treatment was replicated four times. The plastic was cleaned daily to remove dust which would diminish light transmission and removed nightly to permit dew formation on the plants. On a clear day, mid-day intensity measurements of long-wave (300-400 nm) ultraviolet (UV) radiation under the shelters were recorded with a Blak-Ray Ultraviolet Intensity Meter and the average of four readings per treatment were 3.2×10^3, 18.5×10^3, and 26.4×10^3 (± 5.0%) ergs/sec/cm^2 for Mylar, Tedlar, and no plastic, respectively. The total ripe fruit and the number of infected fruit from each treatment was counted and percent diseased fruit calculated.

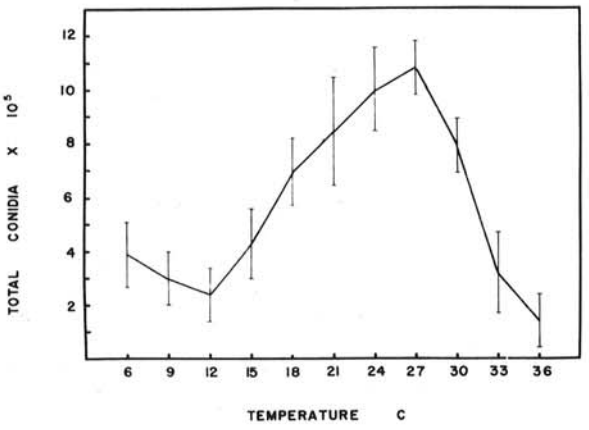

Fig. 3. Effect of temperature on sporulation of *Alternaria alternata* on water agar. Each point represents the mean number of conidia of five replicates. Standard errors are indicated.

The highest incidence of blackmold occurred in the unprotected control plots where 66% of the 1,514 ripe fruit were infected. The Tedlar treatment had 46% of 1,059 ripe fruit infected, and the Mylar treatment, which allowed passage of the least amount of UV radiation, resulted in 35% of its 1,053 fruit infected–a marked reduction in the amount of blackmold.

High ambient temperatures are common during the tomato-growing season in California so the effects of infrared radiation (heat) on blackmold were studied. Thermocouples were placed within the subepidermal tissue of exposed green tomato fruit in the field and temperatures were monitored. Temperature recordings also were taken of exposed fruit which had been treated with Sungard®, a commercially available lime-base sunburn protectant at a rate used commercially. Sungard was applied after the weight of the green fruit had begun to bend down the branches, thereby exposing previously shaded fruit to direct sunlight. The percent infected fruit was determined in the Sungard treated and untreated plots after the fruit had ripened. Each treatment was replicated eight times.

Temperatures in subepidermal tissues of Sungard shaded and unshaded exposed green fruit were compared with ambient temperatures. Fruit temperatures were 10-15 C higher than the ambient temperatures in the afternoon but were lower than ambient temperatures in the early morning hours. Although fruit temperatures were reduced about 5 C by Sungard shading, the percent blackmold was not significantly reduced. In the Sungard treatment, 16% of 798 ripe fruit had blackmold and in the untreated check 17% of the 777 ripe fruit had blackmold.

Predisposition of green fruit to infection and treatments to stimulate fleck expansion.—The resistance of green fruit to *A. alternata* observed in the field and greenhouse perhaps could be altered by exposures of fruit to extremes of temperature and short-wave UV radiation. Therefore, vine-attached 'Hardin Miniature' fruit were subjected to various treatments. Neither heat treatment of fruit at 38 C for various exposure periods, nor short periods at a higher temperature (48 C) prior to inoculation had any effect on green fruit susceptibility or lesion development. Likewise, exposure of fruit to diurnal cycles of 34 and 22 C or 27 and 15 C before and after inoculation, or exposure to a preinoculation 10-day cycle of 4 hours at 10 C and 20 hours at 24 C, had no effect on green fruit susceptibility or lesion development. In addition, fruit irradiated with short-wave (254 nm) UV radiation at a distance of 10 cm for 5-15 minutes before inoculation developed lesions much the same as nonirradiated inoculated fruit. Symptoms produced in all of these treatments were the small shallow lesions and none of the treatments increased the number or severity of the lesions.

Vine-ripened fruit with arrested fleck lesions resulting from inoculation with *A. alternata* while still green, were

subjected to various treatments in attempts to induce lesion expansion. Mechanical injury of the affected tissue by pin pricking had no effect on lesion expansion. Flecks on field-collected ripe fruit failed to expand when surface treated with 0.5% NaOCl prior to placement in a saturated atmosphere for 14 days at room temperature (approximately 25 C) or at 10 C. Lesion size on harvested greenhouse-grown ripe cultivar Hardin Miniature fruit did not increase at 25 C after being subjected to temperatures of 6, 12, 18, 24, 30, and 33 C for 8 days.

Anatomical studies.—Alternaria alternata was readily isolated from the arrested flecks in greenhouse-ripened fruit. Since attempts to stimulate enlargement of these flecks had failed, a histological study of the arrested flecks was made to determine whether a physical barrier around the lesion was present. Flecks on ripe Hardin Miniature fruit that had been initiated by green fruit inoculation under greenhouse conditions, were excised, killed and fixed in formalin-acetic acid, dehydrated in a tertiary butyl alcohol series, passed through paraffin oil, and infiltrated with Fisher Tissuemat. Sections cut at a thickness of 12-15 μm were stained with saffranin-fast green (5).

Fungal mycelium was observed primarily intercellularly in the epidermis and subepidermal tissue. Necrosis had not extended beyond the third layer of cells below the surface in any of the lesions examined. No cicatrix formation, suberized, or thickened cell walls were apparent, indicating no visible physical barrier to fungal advancement.

Factors affecting inoculum production and dispersal.—A. alternata was observed to sporulate on various substrates in tomato fields; i.e., any senescent or nonliving plant material, dead areas in tomato leaflets resulting from salt toxicity, healthy leaflets covered by aphid honeydew, and blackmold lesions on tomato fruit.

The effect of temperature on sporulation was determined in the laboratory. Petri dishes of Difco water agar (20 ml/dish) were inoculated with 0.3 ml of a conidial suspension, 10^6 conidia/ml, of *A. alternata* (isolate $SSLC_3$). The conidia were distributed over the surface of the agar with a glass rod and the dishes were incubated at room temperature. After 48 hours, five dishes were placed in each of 11 paper bags and incubated 12 days in darkness at temperatures ranging from 6 C to 36 C at 3 C increments. To correct for spores added as inoculum, and produced within the first 48 hours, spores from five culture dishes were harvested and the average number of conidia obtained from them was subtracted from the numbers of conidia obtained at the termination of the experiment. The numbers of conidia per dish were determined by flooding the dishes with 5 ml of water and gently rubbing the surface of the colony with a bent glass rod. Conidial concentration was determined with a haemocytometer and the total conidia in each 5 ml was calculated.

Temperatures in the 21-30 C range were most favorable for sporulation (Fig. 3). A marked reduction in sporulation occurred at or below 15 C and at temperatures $\geqslant$ 33 C. The optimum temperature for sporulation was 27 C.

In a field study using a Burkard spore trap, most of the conidia of *A. alternata* were trapped during the afternoon hours demonstrating a diurnal periodicity. Average

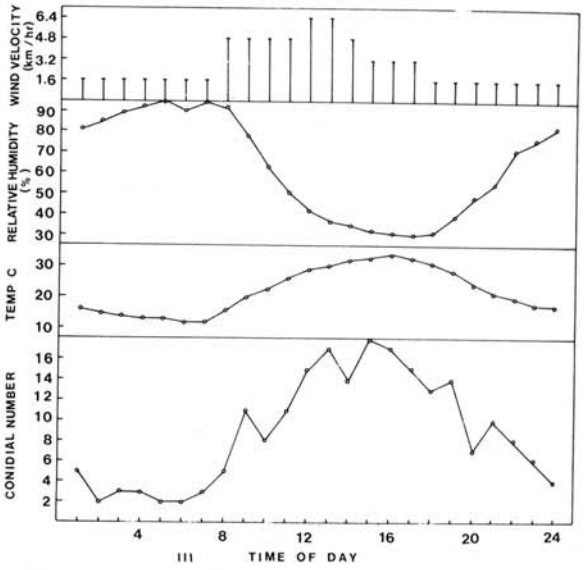

Fig. 4. Weather parameters that affect the release of conidia of *Alternaria alternata*: wind velocity (km/hour), percent relative humidity, temperature, and number of conidia trapped. Each plot is based on an average of 10 consecutive days beginning 4 September 1971 in a tomato field at Davis, California. Time indicated is Pacific Daylight Saving Time.

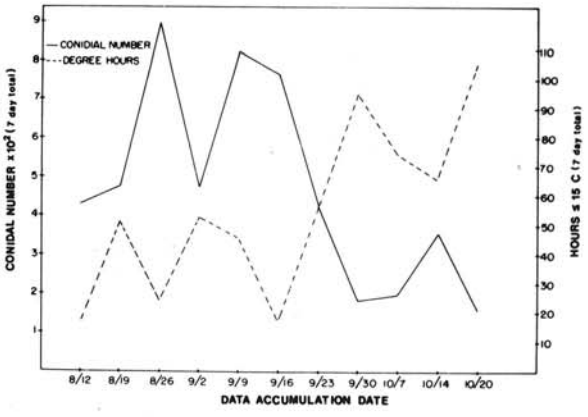

Fig. 5. Inverse relationship of conidial numbers and the number of hours at or below 15 C. Values represent 7-day totals for numbers of conidia trapped and number of hours at or below 15 C. Data accumulation dates represent the last day of a 7-day period for which data were recorded in 1971.

hourly data for conidial numbers, temperature, percent relative humidity and wind velocity for 10 consecutive days from 4 September to 13 September, 1971 are given in Fig. 4. Highest conidial numbers were correlated with maximum temperatures, minimum humidity, and maximum wind velocity.

There was considerable seasonal variation in numbers of *A. alternata* conidia trapped during 1969, 1970, and 1971. The highest numbers of conidia were trapped in August and early September. A decrease in the number of conidia trapped was observed after mid-September, al-

though this was usually the time when blackmold was most prevalent. Based on the above temperature studies, a relation between hours at or below 15 C and number of conidia trapped was determined from data collected in 1971 (Fig. 5). The results indicated that the number of conidia trapped decreased as the season progressed, and there was an inverse correlation between numbers of conidia and low temperatures.

DISCUSSION.—Dew formation on the surface of tomato fruit is the most critical requisite for development of blackmold lesions in the absence of rain. Lesions occur only on the upper surface of fruit not protected by a leaf canopy. Dew was deposited only on the upper surface of exposed fruit but generally at temperatures below optimum for rapid germination of conidia. However, glucose and fructose in exudates on the fruit surface and dissolved in the dew partially offset the depressing effect of the suboptimal temperature by stimulation of conidial germination. This dew deposition pattern accounts for the lesion distribution. Thus these studies do not support suggestions that differential inoculum deposit (33) or sun exposure (4) account for the lesion distribution. Although there appeared to be an effect of UV radiation in the field on disease incidence and severity, greenhouse studies showed that they were not directly attributable to the influence of UV light.

Infections of green fruit by *A. alternata* produce tiny quiescent lesions that are of minimal importance in the development of severe rot in ripe fruit as opposed to Koepsell's (13) findings. We found the large severe lesions developed from infections that occurred after the fruit ripened and were responsible for most of the losses incurred in processing tomatoes. Why the quiescent green fruit lesions failed to expand even though living mycelium was present in them was not determined. Conidia of *A. alternata* were trapped throughout the growing season, but their numbers decreased during the last portion of the season while the incidence of blackmold increased. Most conidia were trapped in midsummer (August and early September) as previously found (8, 12, 25). However, the marked decrease in the numbers of conidia toward the end of the growing season (late September and October) contrasts with reports by Rotem (22) that indicated maximum dispersal of *A. porri* and *A. tenuis* conidia during the final stages of early blight disease in tomato and potato in March. Rotem (22) found no detectable influence of temperature on spore dispersal; however, we found that cool temperatures decreased production of conidia.

An increase in disease incidence with a reduction in inoculum levels late in the growing season is unexplained. A factor influencing an increase in blackmold may be extended periods of dew duration that provide water for spore germination at the low temperatures encountered. Low temperatures (5 - 10 C) may predispose fruit to infection as shown by McColloch and Worthington (16) for *A. alternata*. Thus longer periods of dew, low temperatures, and longer periods of exposure of fruit because of slower ripening combined with the stimulatory effects of sugars in the exudates on the fruit apparently offset the effects of lower inoculum levels.

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