Dispersal of Conidia of Botrytis cinerea in Tomato Fields

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ABSTRACT

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Conditions influencing dispersal of conidia of *Botrytis cinerea* were studied in two fields of staked tomato plants near Carpinteria and Chula Vista, California. Conidia were trapped with a Burkard spore trap (10 liters/min) throughout the growing season. In one field, trapped conidia averaged less than 50 per day when disease incidence was less than five decayed fruit per 15 plants, but increased fivefold when disease incidence was 20-30 decayed fruit per 15 plants during the last month of the growing season. Circadian periodicity in spore dispersal was observed. Maximum concentrations of spores occurred about midday. This circadian periodicity was positively correlated to changes in temperature and wind velocity and negatively correlated to changes in percent relative humidity and presence of dew. Wind velocities up to 9.6 km/hr above the canopy of the tomato field resulted in wind velocities of less than 0.5 km/hr at sites of stem cankers located at the bases of the plants. The incidence of disease along a disease gradient from a line source of inoculum showed that the spread of inoculum and subsequent disease incidence was limited to plants within 8 m of the inoculum source. In a wind tunnel, few conidia were dispersed from a 6-cm-diameter, sporulating colony grown on potato-dextrose agar that was exposed to wind velocities of about 0.4 km/hr. Greater numbers of conidia were released and dispersed from the same colony on agar exposed to higher wind velocities. Spread of conidia of *B. cinerea* to adjacent plants by air is probably limited by the low wind velocities within the tomato canopy which would affect dispersal of released conidia from sites of sporulation.

Additional key words: gray mold, disease incidence, environmental conditions.

Botrytis cinerea Pers. causes gray mold of tomato (*Lycopersicon esculentum* Mill.) and is a common problem for tomato growers especially in the coastal areas of California. Infections of leaflets and attached blossoms occur early in the growing season and result in stem cankers that ultimately may girdle the stem and kill the entire plant (2). *Botrytis cinerea* sporulates profusely on these cankers and other infected tissues.

Dobbs (4) classified *Botrytis* as a "spore-shedder," meaning that most spores are shed when sporulating surfaces are subjected to gentle winds. Conidia of *Botrytis* sp. are common components of the spore population of air (3, 5, 7, 8, 9, 10, 12). Several workers also have reported a diurnal periodicity, with the highest number of conidia being trapped at midday (3, 8, 12). Airborne conidia of *B. cinerea* have been recorded in association with disease in strawberry fields (6, 10), raspberry plantations (8), and grape vineyards (1, 3, 9). Miller and Waggoner (10) found low levels of airborne conidia throughout the fruiting period, even though there were as many as 10 infected fruit per 0.3 m of row in a 0.2-hectare strawberry planting. They concluded that most infections by *B. cinerea* originated from a nearby source of inoculum. Jarvis (8) found that the number of airborne conidia trapped in a raspberry plantation increased when the fruit ripened. Sporulation occurred on the fruit in the field and on the fleshy receptacles after harvest. He (8) concluded that the limiting factors for release and dispersal of conidia were temperature and relative humidity. He (6) showed that changes in relative humidity were responsible for release of conidia from their attachments to the conidiophore, but these changes did not result in dispersal of the released conidia from the conidiophore. Some form of mechanical shock or air movement was needed to disperse released conidia. Jarvis also pointed out the importance of splash dispersal of conidia of *B. cinerea* during rainy periods (7). Corbaz (3) and Lehoczky (9) found in vineyards that the number of conidia of *B. cinerea* trapped increased throughout the growing season so that the highest numbers occurred during the late summer and fall. Conidia also were more abundant in wet than in dry years (3). Bulit and Verdu (1) found that there was an increase in the number of conidia of *B. cinerea* trapped in a vineyard in spring during bloom which corresponded to the period of host susceptibility.

The purpose of trapping conidia of *B. cinerea* in fresh market tomato fields was to determine (i) if the conidia

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were present throughout the growing season; and (ii) what influence disease levels and environmental factors had on the numbers of conidia in the air. This information is needed to develop a more effective control program.

MATERIALS AND METHODS

Dispersal of conidia in tomato fields.—Conidium dispersal was monitored in vine-ripe tomato fields in the coastal areas of Carpinteria and Chula Vista, California. These areas were selected because of the common occurrence of gray mold there. Temperature, relative humidity, wind velocity, rainfall, dew duration, and the number of spores in the air over fields were monitored from 9 July through 4 December 1974 at Carpinteria and from 25 June through 9 July 1975 in Chula Vista. Cultivars 6718, 6339 (Peto Seed, Woodland, CA 95695), and 428 (Ferry-Morse Seed Co., San Juan Bautista, CA 95045) were grown at Carpinteria while cultivar 6718 was grown at Chula Vista.

A weather station consisting of a standard weather shelter, hygrothermograph (H311, Weather Measure Corp., Sacramento, CA 95652), recording rain gauge (P501-1, Weather Measure Corp., Sacramento, CA 95652), leaf-wetness recorder ("Dr. DeWit," Valley Stream Farm, Orno, Ontario, L0B 1M0, Canada), and an anemometer (02AM100 mechanical anemograph, Kahl Scientific Instrument Corp., El Cajon, CA 92020) were used in the tomato fields during the monitoring. A Burkard 7-day recording volumetric spore trap (Burkard Mfg., Co., Ltd., Rickmansworth, Herts, England) was used to monitor the spore content of the air. The intake orifice was approximately 0.5 meters above the canopy and sampled 10 liters of air per minute throughout the monitoring period. The orifice always faced into the wind. Mounted tapes were directly scanned under ×720 magnification for conidia of *B. cinerea*. Identification was based on conidium size, shape, color, and surface texture.

Dispersal of conidia from a ring source of sporulating stem cankers.—From 9 July through 23 July 1975 the Burkard spore trap at Chula Vista was surrounded with cankered tomato stems on which *B. cinerea* was sporulating. This provided a ring source of conidia of *B. cinerea* (Fig. 1-A) to determine the importance of environmental factors on spore dispersal. Environmental data were obtained as before from instruments placed in the tomato field adjacent to the trap.

Wind velocities in the field.—The relationship of the wind velocity 50 cm above the tops of a tomato canopy (150 cm above the soil) to the wind velocity at the main branching point of the stem, 20-30 cm above the soil was determined in fields at Chula Vista. Average wind velocities above the canopy were determined for 10-min intervals with an air flow meter (Model W-131, Weather Measure Corp., Sacramento, CA 95652) that faced into the wind. Cumulative air flow for the 10-min sample period was used to determine average velocity for that 10-min period. Simultaneous wind velocity measurements were taken at the main stem branch of the tomato plant every 15 sec for the same 10-min period with a thermoanemometer ("Alnor" type 8500, Alno Instrument Co., Chicago, IL 60690). The thermoanemometer probe was placed 5 mm above the stem with the element parallel to the long axis of the stem so that the air flow across the stem was measured. The average of the 40 recordings per 10-min period were compared to the air flow above the canopy. Thirteen such tests were made throughout a 2-day period.

Disease incidence at different distances from a line source of inoculum under field conditions.—A plot with 29 cultivars or pedigreed breeding lines was established on the west side of a grower's field near Carpinteria, California. On 11 June, 685 plants were transplanted into adjacent rows on 1.5-meter centers (108 plants/row, spaced 46 cm apart). These plants received no fungicides throughout the growing season and *B. cinerea* was well established in the plot by the end of September. By 3 December, 56% of the plants had been killed by stem cankers, 28% had 75% of the foliage blighted, 13% had 50% of the foliage blighted, and 3% of the plants had 25% of the foliage blighted. *Botrytis cinerea* was sporulating profusely on the dead or dying plants.

The grower had direct-seeded tomato seed (cultivar 428) on 18 May in rows on 1.5-m centers 1.5 m east of, and

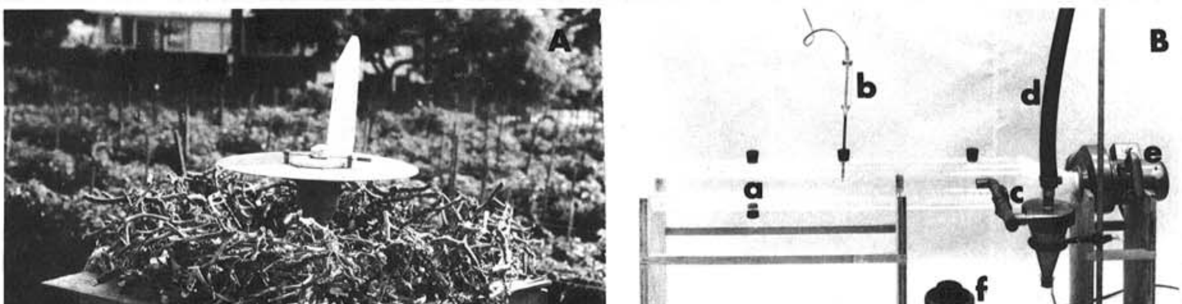

Fig. 1-A, B. Instruments used to study spore dispersal of *Botrytis cinerea*. **A)** Arrangement of Burkard spore trap and tomato stems with cankers bearing conidia of *B. cinerea*. **B)** Wind tunnel used to measure spore dispersal from colonies grown on potato-dextrose agar. a) Specimen stage, b) thermoanemometer, c) cyclone spore trap, d) hose to vacuum for spore trap, e) suction motor, and f) variable rheostat for suction motor.

parallel to the rows in the plot and perpendicular to the prevailing wind. The plants were thinned to one plant every 46 cm, with 108 plants/row. The grower applied chlorothalonil (Bravo 6F) at 578 ml/hectare about twice each month from early September through December. The incidence of disease in the grower's field was determined by counting the number of dead plants in each row east of the plot (downwind) on 3 December.

Effect of wind velocity on the release and dispersal of conidia.—The effect of wind velocity on the dispersal of conidia of *B. cinerea* was studied in a wind tunnel. The tunnel (Fig. 1-B) was constructed of clear acrylic tubing with an inside diameter of 10.8 cm. The specimen stage was located 30.5 cm from the open end of the tube and the intake orifice for a cyclone spore trap was located 76.2 cm from the specimen stage. An isolate of *B. cinerea* isolated from a tomato stem canker from Carpinteria, California in 1974 was grown for 7 days in 6-cm-diameter plastic plates of potato-dextrose agar incubated at 20 ± 1 C under continuous light (one fluorescent General Electric F48T10CW lamp, 2,700 lux). The sporulating colonies then were removed intact from the petri plates and placed one at a time on the stage in the wind tunnel. A cyclone spore trap (11) was used to sample the spores released and dispersed from the sporulating colonies. The flow of air through the spore trap was constant at approximately 200 liters/min. According to K. G. Tate (*personal communication*) more than 99% of the conidia of *Monilinia fructicola* (Wint.) Honey entering the spore trap are retained by the trap at this level of air flow. Wind velocities up to 1.6 km/hr were obtained using the spore trap vacuum alone, and velocities greater than 1.6 km/hr were obtained by using a suction motor at the end of the wind tunnel. Each sporulating colony was subjected to consecutive 2-min exposures to air speeds of 0.4, 1.6, 4.8, 8.0, and 12.8 km/hr. Three samples were run per experiment and the experiment was repeated. The temperature and relative humidity of the air during the test were 22 ± 1 C and 35-40%, respectively.

The conidia were collected in 50 ml of water and conidia which may have been retained within the trap were removed by rinsing the trap and intake nozzle with 50 ml of water after each test. These suspensions were filtered through a millipore filter (0.22 μm pore size) to recover the trapped conidia. The filter then was placed in a test tube with 2 ml of water and vigorously shaken in a Vortex mixer. The concentration of conidia in this 2-ml suspension was determined using a haemacytometer. The total number of conidia dispersed from the source was calculated after correcting for the volume of air actually sampled in relation to the volume of air moving through the tunnel assuming a trapping efficiency of 99%. Air velocities inside the tunnel were measured with a thermoanemometer.

RESULTS

Dispersal of conidia in tomato fields.—At Carpinteria conidia of *B. cinerea* were present in the air throughout the growing season. The number of conidia trapped were

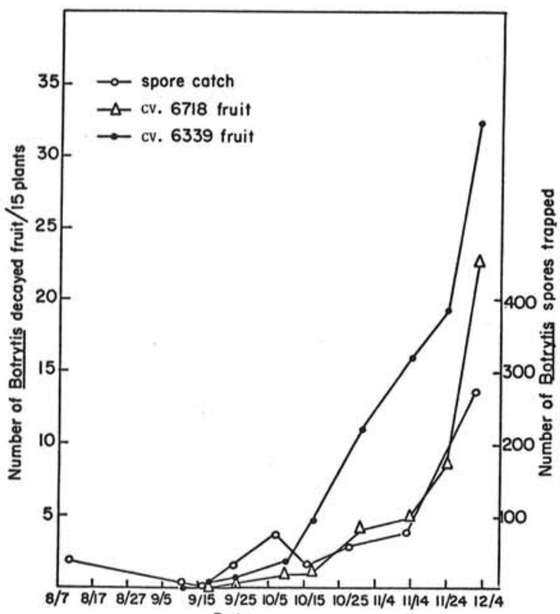

Fig. 2. Relationship between the number of conidia of *Botrytis cinerea* trapped and the incidence of cultivar '6718' and '6339' tomato fruit decayed by *B. cinerea* at Carpinteria, California in 1974.

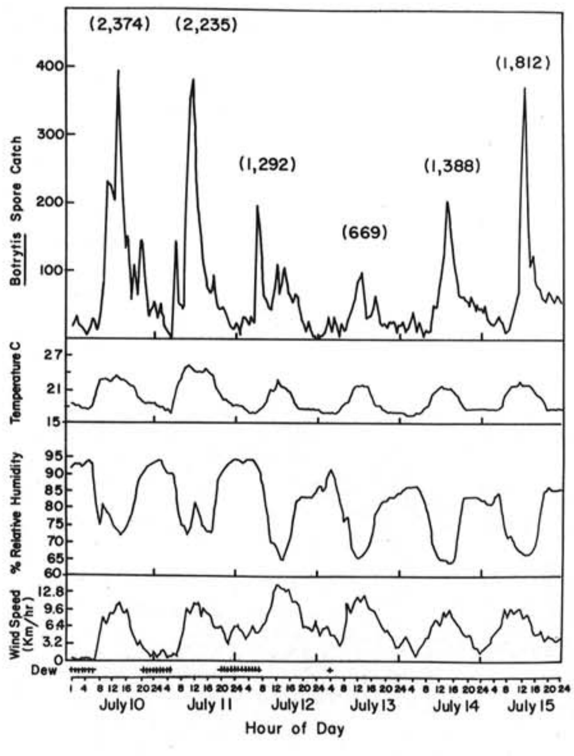

Fig. 3. Circadian periodicity of trapped conidia of *Botrytis cinerea*. Hourly number of trapped conidia, temperature, percent relative humidity, wind speed, and presence of dew from 10 July through 15 July 1975. Numbers in parentheses indicate total number of conidia trapped each day. Time indicated is Pacific Daylight Savings Time.

low, but as the amount of disease increased during the season, the number of conidia trapped per day showed a slight increase (Fig. 2). None of the plants at Carpinteria were killed by stem cankers. In Chula Vista the incidence of disease was higher than at Carpinteria (7% of the plants had been killed by 25 June), but the levels of conidia trapped were low. During the 14 days of trapping, the highest number of conidia trapped per day was 75 on 26 June.

TABLE 1. Correlation of environmental factors and airborne conidia of *Botrytis cinerea* trapped in each of six consecutive 24-hr days starting 10 July

Day	Correlation with spores trapped[a]			
	Temperature (C)	RH (%)	Wind velocity (m/sec)	Presence of dew
1	0.69***	−0.67***	0.77***	−0.59**
2	0.67***	−0.57**	0.62***	−0.56**
3	0.27	−0.21	0.30	−0.07
4	0.74***	−0.73***	0.70***	−0.11
5	0.68***	−0.73***	0.74***	
6	0.46*	−0.51*	0.57**	
Mean	0.51***	−0.37***	0.41***	−0.20*

[a]Correlation (r) of environmental factors (Fig. 2-B) on an hourly basis with spores trapped from a ring source of tomato stems with cankers caused by *B. cinerea*. Probabilities of significance designated by ***, **, and * are $P = 0.001, 0.01$, and 0.05, respectively.

Dispersal of conidia from a ring source of sporulating stem cankers.—The circadian periodicity of conidia of *B. cinerea* in the air near a source is shown in Fig. 3. There was a significant positive correlation between the number of conidia trapped in 1-hr periods and temperature and wind velocity. There was a significant negative correlation between the number of conidia trapped in 1-hr periods and percent relative humidity (RH) and the presence of dew (Table 1).

Effect of wind velocity on the release and dispersal of conidia.—Some dispersal of conidia occurred in the wind tunnel with an air velocity of only 0.4 km/hr but much greater numbers were dispersed from the same source exposed to higher wind velocities (Fig. 4-A). There was a positive correlation between the wind velocity above the tomato canopy and the wind velocity inside the canopy at the main branch of the stems (Fig. 4-B) where most stem cankers were located. Although the two wind velocities

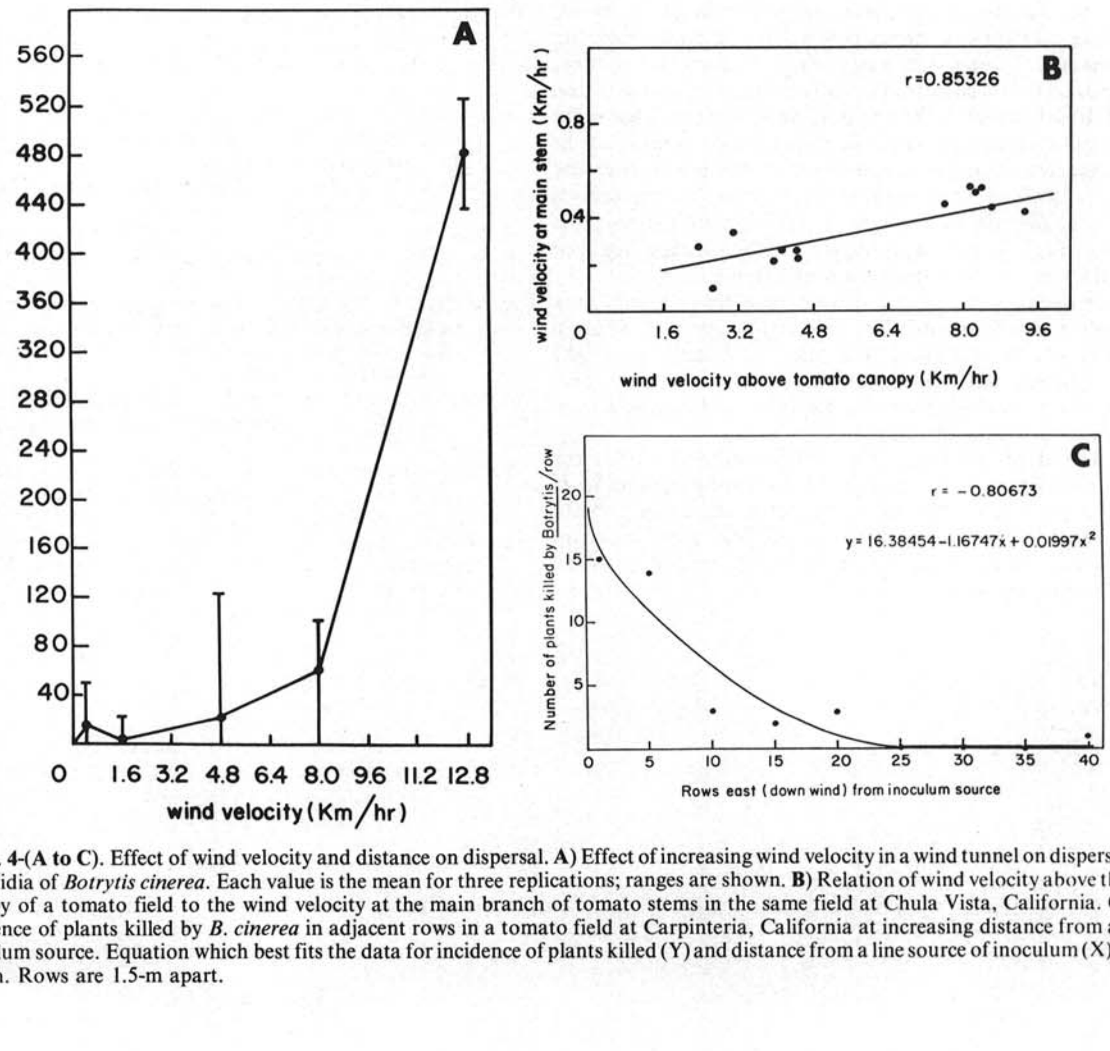

Fig. 4-(A to C). Effect of wind velocity and distance on dispersal. **A)** Effect of increasing wind velocity in a wind tunnel on dispersal of conidia of *Botrytis cinerea*. Each value is the mean for three replications; ranges are shown. **B)** Relation of wind velocity above the canopy of a tomato field to the wind velocity at the main branch of tomato stems in the same field at Chula Vista, California. **C)** Incidence of plants killed by *B. cinerea* in adjacent rows in a tomato field at Carpinteria, California at increasing distance from an inoculum source. Equation which best fits the data for incidence of plants killed (Y) and distance from a line source of inoculum (X) is shown. Rows are 1.5-m apart.

were significantly correlated (r = 0.85), increases in wind velocity above the canopy from 2 to 9.6 km/hr only increased the velocity from 0.1 to 0.5 km/hr at the site in the canopy where the fungus sporulated on stem cankers.

Disease incidence at different distances from a line source of inoculum under field conditions.—There was a significant negative correlation between the number of dead plants per row and the distance of the row from the variety plot (r = −0.81). There was a sharp decrease in the number of dead plants per row beyond the fifth row or 8 m from the cultivar plot (Fig. 4-C).

DISCUSSION

Conidia of *B. cinerea* were present in the tomato fields throughout the growing season. In the field at Carpinteria (Fig. 2-A) there was an increase in the number of conidia trapped per day with increasing incidence of decayed fruit late in the growing season, as was found by Jarvis with raspberry (9). However, at Chula Vista, where the grower had lost 7% of his plants from stem cankers at the time of trapping, very few conidia were trapped per day. This suggested that in spite of large amounts of sporulating material within the canopy there was only limited dispersal of conidia of *B. cinerea* into the air above it (Fig. 2-B).

The number of conidia trapped near the exposed cankered tomato stems (spore trap surrounded with cankered stems) was much greater than the number trapped from the field. This was caused by a combination of three factors: (i) there was an immediate increase in the amount of conidia available for dispersal because of the concentrated source of sporulating material; (ii) the wind velocity affecting dispersal in this experiment approached that of the wind velocity above the tomato canopy, and (iii) placing the trap close to the source reduced sedimentation and dispersion of conidia.

It seems that the leaf canopy in a tomato field may reduce the dispersal of conidia from sporulating stem cankers by reducing the air movement past the sporulating surface. Some dispersed conidia are also probably removed from the air by impaction and do not clear the tomato canopy.

The data shown in Fig. 3-C demonstrate that the disease incidence decreased with increasing distance from a line source of inoculum. Except when a new tomato field is planted adjacent to an old field with abundant sporulation by *B. cinerea*, this level of inoculum would not normally occur.

Since conidia of *B. cinerea* are present, and environmental conditions in these coastal tomato-growing areas are favorable for infection and disease development it is important to protect the vines and fruit from infection. Fungicides can protect plants from infection (1) and reduced infection would result in less disease which results in lower inoculum levels. This indicates that applications of fungicides during the early part of the growing season would be more important than applications during the latter part of the season because of their ability to delay the onset and development of disease and thus keep inoculum levels low.

LITERATURE CITED

1. BULIT, J., and D. VERDU. 1973. Variation journaliere et annuelle de La sporee Aerienne du Botrytis cinerea Pers. dans un vignoble. Ann. Phytopathol. 5:319 (Abstr.).
2. CHASTAGNER, G. A., J. M. OGAWA, B. T. MANJI, and T. T. MATSUMOTO. 1977. Botrytis cinerea stem canker development and control on vineripe tomatoes in California. Proc. Am. Phytopathol. Soc. 4:114.
3. CORBAZ, R. 1972. Investigations in fungal spores trapped in a vineyard. Phytopathol. Z. 74:318-328.
4. DOBBS, C. G. 1942. On the primary dispersal and isolation of fungal spores. New Phytol. 41:63-69.
5. HIRST, J. M. 1959. Spore liberation and dispersal. Pages 529-538 *in* C. S. Holton, ed. Plant pathology: problems and progress, 1908-1958. Univ. of Wisconsin Press, Madison. 588 p.
6. JARVIS, W. R. 1960. An apparatus for studying hygroscopic responses in fungal conidiophores. Trans. Br. Mycol. Soc. 45:315-319.
7. JARVIS, W. R. 1962. Splash dispersal of spores of Botrytis cinerea Pers. Nature (Lond.) 193:599.
8. JARVIS, W. R. 1962. The dispersal of spores of Botrytis cinerea Fr. in a raspberry plantation. Trans. Br. Mycol. Soc. 45:549-559.
9. LEHOCZKY, J. 1972. Biology of gray mold (Botryotinia fuckeliana), disease cycle of gray rot on grapevine and basic requirements of an effective cluster protection. Szolo-es Gyumolestermesztes 8:217-251.
10. MILLER, P. M., and P. E. WAGGONER. 1957. Dispersal of Botrytis cinerea among strawberries. Phytopathology 47:24-25 (Abstr.).
11. OGAWA, J. M., and H. ENGLISH. 1955. The efficiency of a quantitative spore collector using the cyclone method. Phytopathology 45:239-240.
12. SREERAMULU, T. 1959. The diurnal and seasonal periodicity of spores of certain plant pathogens in the air. Trans. Br. Mycol. Soc. 42:177-184.