The Role of Dew and Temperature in the Epidemiology of Botrytis Leaf Blight of Onion

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ABSTRACT

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Numerous leaf blight lesions developed within 48 hr on intact onion leaves which remained wet continuously for at least 6 hr in a dew chamber at 20 ± 1 C following inoculation with conidia of *Botrytis squamosa*. Lesion numbers increased significantly for each additional 2-hr increment of leaf wetness when tested to 18 hr. When conidia were brushed onto dry leaf surfaces which remained dry in an atmosphere of 92% relative humidity, lesions did not develop. Leaf blighting increased for each 12-hr increment in dew period from 12 to 60 hr after inoculation. Lesion numbers and leaf blighting were significantly greater on older leaves than on younger leaves. Lesions developed over a range of 9 to 23 C after exposure of plants to a leaf wetness period of 40 hr at constant temperatures ranging from 7 to 30 C. Germination of conidia was maximum at 15 C, and it occurred over the range of 12-27 C; growth in culture was maximum at 24 C, and it occurred from 9-31 C.

Additional key words: *Allium cepa*, etiology, *Botrytis squamosa*.

Botrytis leaf blight of onion (*Allium cepa* L.), which is caused by *Botrytis squamosa* Walker, is characterized by leaf spotting followed by a progressive die-back from the leaf tips. Although a number of investigators have established the causal relationship between *B. squamosa* and leaf spotting followed by tip necrosis (4, 5, 6, 8, 13), the relationships between environmental factors and symptom development under controlled conditions still is not clearly defined. Page (7) noted that wet weather followed by dry, hot weather favored development of the disease. He induced the disease (8) by exposing plants inoculated with *B. squamosa* to an alternating environment of 16 hr of light at 25 C and 8 hr of darkness at 12 C. This change in temperature allowed condensation to occur on the leaves, and he suggested that this was a prerequisite for infection. Page also reported that 20 C was the optimum temperature for mycelial growth of the pathogen (8). Segall (10) and Segall and Newhall (11) observed that relative humidity (RH) close to 100% for at least 24 hr was necessary for the leaf spotting phase and that temperatures above 27 C (80 F) were required for the leaf blighting phase. McLean and Sleeth (6) reported that precipitation, high humidity, and cool cloudy weather favored disease development. Page (7, 8) and McLean and Sleeth (6) noted that wilting accompanied spotting on the outer leaves, whereas the inner leaves became spotted but did not wilt.

In the literature to date, moisture has been the primary factor associated with Botrytis leaf blight development. Our study was conducted to determine (i) the relationship of dew to the development of Botrytis leaf blight symptoms, (ii) the importance of leaf position to symptom development, and (iii) the influence of temperature on spore germination and mycelial growth of the pathogen and lesion development. A preliminary report of this work has been published (12).

MATERIALS AND METHODS

Isolates, culture, and inoculum production.—*Botrytis squamosa* isolates obtained from nature sporulate poorly in culture and require special treatment to induce sporulation (1). Therefore, the predominant isolate used in these studies was a mutant (64a, which was equal to the wild type in virulence) that sporulates rapidly (2). Three wild-type isolates, two from Orange County, New York (62-10 and 69-13) and one from Long Island, New York (66-6), were compared with 64a in two experiments. All cultures were maintained on test tube (2.5 × 20 cm) slants that contained 10 ml of a complete medium (2).

For growth/temperature studies, 5-mm disks were cut from the perimeter of 3-day-old cultures of each of the four isolates growing on 15 ml of potato dextrose agar (PDA) in 9-cm-diameter petri dishes. These disks were transferred to media in plates and flasks used in the temperature studies which are described later.

Inocula for studies on lesion production and blighting were produced by growing isolate 64a on 10 ml of the complete medium in test tube slants. Conidial suspensions were obtained by pouring a total of 20 ml of sterile distilled water in 5-ml aliquots over the surface of

sporulating 10-day-old cultures, scraping the surface with a transfer needle, and then filtering the spore suspension through a double layer of Kimwipes (type 900-S, Kimberly-Clark Corporation, Neenah, WI 54956) to remove dislodged conidiophores, mycelium, and agar fragments. The conidia were counted in a hemacytometer, and the concentration was adjusted with distilled water. Conidial suspensions were atomized at 1.66 atmospheres (25 psi) line pressure onto onion plants in a dew chamber.

The dew chamber.—To produce continuous free moisture on leaves, a dew chamber was built and placed inside a reach-in controlled environment chamber (Fig. 1-A). The dew chamber (64 × 66 × 72 cm) was constructed from painted white pine framing (2 × 4 cm) covered with 0.102-mm (4-mil) polyethylene. The base was covered with 6-mm mesh (0.25-inch) galvanized screening and set over a water bath. A submersible pump in the bath maintained water circulation at a uniform temperature, and aided evaporation. A small fan (1,250 rpm, 0.5 amp, 1/100 HP) with air flow directed over the water bath operated continuously to increase evaporation and prevent temperature stratification within the dew chamber. This maintained the water vapor of the air within the dew chamber near saturation. Temperature on the outside of the dew chamber was regulated by the controls for the reach-in chamber. Temperature within the dew chamber was regulated by a heating coil and thermostat located in the water bath. By setting the water bath temperature 3 to 4 C higher than the temperature of the reach-in chamber, a radiation flux was established from within to the outside of the dew chamber, with lights either on or off. This caused the leaves to be cooled below the dew point and resulted in dew formation (Fig. 1-B). The temperature of the water bath and the ambience within the dew chamber were monitored continuously by thermocouple sensors.

The dew studies were conducted at 20 ± 1 C and photoperiod of 14 hr of fluorescent light (1,937 lux) and 10 hr of darkness. Different dew periods were achieved by removing groups of plants from the dew chamber at different times after inoculation; upon their removal, they were held in a walk-in chamber at 70% RH, 20 ± 1 C, and with 14 hr of light (21,520 lux) and 10 hr of darkness.

Analyses.—The data from the dew studies were analyzed by an analysis of variance after fitting the data to covariance models described by Searle (9). Computations were performed by using computer program BMDO4V (3).

Dew and pathogenesis.—In the first experiment, conidia were brushed from the surface of a sporulating culture of isolate 64a onto dry leaves (third leaf only) of two different plants. A third plant was used as a noninoculated control. The three plants then were exposed to 48 hr of continuous dew at 20 ± 1 C with alternating 14 hr of light (21,520 lux) and 10 hr darkness. In the second experiment, the same procedure was followed except that 92% RH was substituted for continuous dew.

Dew period and lesion production.—The relationship between duration of dew following inoculation with isolate 64a and numbers of lesions was investigated using 2-mo-old plants (cultivar Elite) grown from seed. In one experiment, duplicate plants were exposed to 0, 3, 6, 9, and 12 hr of dew immediately following inoculation with a water suspension of 10^4 conidia in 5 ml per plant. Two noninoculated plants were exposed to 12 hr of dew. Lesions were counted on the three innermost leaves of each plant 48 hr after inoculation.

In a second experiment, triplicate plants were exposed to 10, 12, 14, 16, and 18 hr of dew immediately following inoculation with 6×10^4 conidia in 3 ml per plant. Lesions were counted on 20-cm lengths of leaf measured from the leaf tip for the four innermost leaves of each plant.

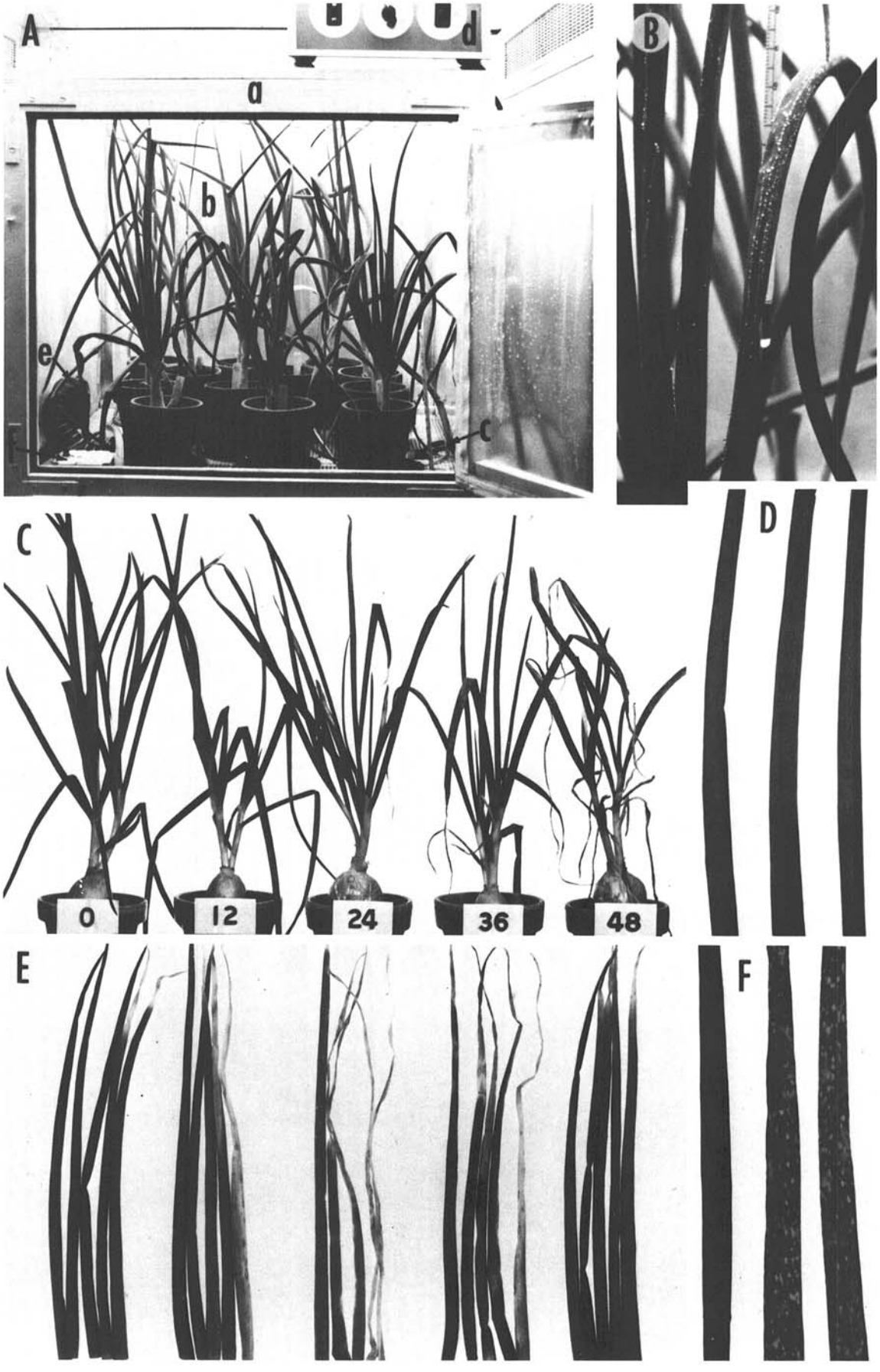
Dew period and blighting.—Two, 2-wk-old plants from sprouted bulbs (cultivar Spartan Banner) were exposed to 0, 12, 24, 36, and 48 hr of dew immediately following inoculation with 1.25×10^5 conidia in 2.5 ml per plant. Two noninoculated plants were exposed to 48 hr of dew. The amount of blighting on each leaf, beginning with the center through the fifth leaf, was recorded 72 hr after inoculation.

Dew period, blighting, lesion production, and leaf position.—The relation between the dew period immediately following inoculation, lesion production, blighting, and leaf position was investigated by using 2-mo-old onion plants (cultivar Elite) grown from seed. After inoculation with 8×10^4 conidia in 4 ml per plant, plants in triplicate were exposed respectively to 12, 36, 60, and 84 hr of continuous dew. Three noninoculated plants were exposed to 84 hr of continuous dew. Following the respective exposures, plants were removed from the dew chamber and held in the walk-in chamber until the plants exposed to 84 hr dew were removed. Then total leaf length and blighted length were measured, and the total number of lesions was counted on five leaves, starting with the center leaf of each plant.

Temperature and mycelial growth.—The effect of temperature on growth of the four isolates was studied over the range of 3-33 C at 3-C intervals. In one experiment, 5-mm disks (described earlier) were transferred to the centers of 9-cm diameter petri dishes containing 15 ml of PDA; colony radius (six replicates) was measured after 3 days. In an other experiment 5-mm disks were transferred to 30 ml of potato-dextrose broth in 250-ml Erlenmeyer flasks; mycelium dry weight (four replicates was measured after 5 days.

Temperature and conidial germination.—Duplicate

Fig. 1-(A to F). Dew as a factor in lesion production and blighting of onion by *Botrytis squamosa*. **A)** The dew chamber: (a) shown within the reach-in controlled environment chamber; (b) onion plants placed in the dew chamber; (c) heating coil; (d) thermostat for the water bath; (e) fan; (f) submersible pump. **B)** Onion leaves covered with dew. **C)** Leaf blighting after subjecting plants to 0-48 hr of dew following inoculation with conidia of *B. squamosa*. **D)** Lack of lesions on two leaves on the right from plants held at 92% RH for 48 hr following inoculation by brushing conidia of *B. squamosa* onto intact plants. Control leaf is on the left. **E)** Leaf blighting following inoculation with *B. squamosa* and exposure to 12, 36, 60, 84, and 84 hr (the latter a noninoculated control) of dew, respectively. The youngest leaf is on the left and the older leaves (increasing in age) are to the right in each set of five leaves. **F)** Leaf spotting after exposing leaves (intact) to 48 hr of continuous dew. Conidia of *B. squamosa* were brushed on the two leaves on the right, control leaf is on the left.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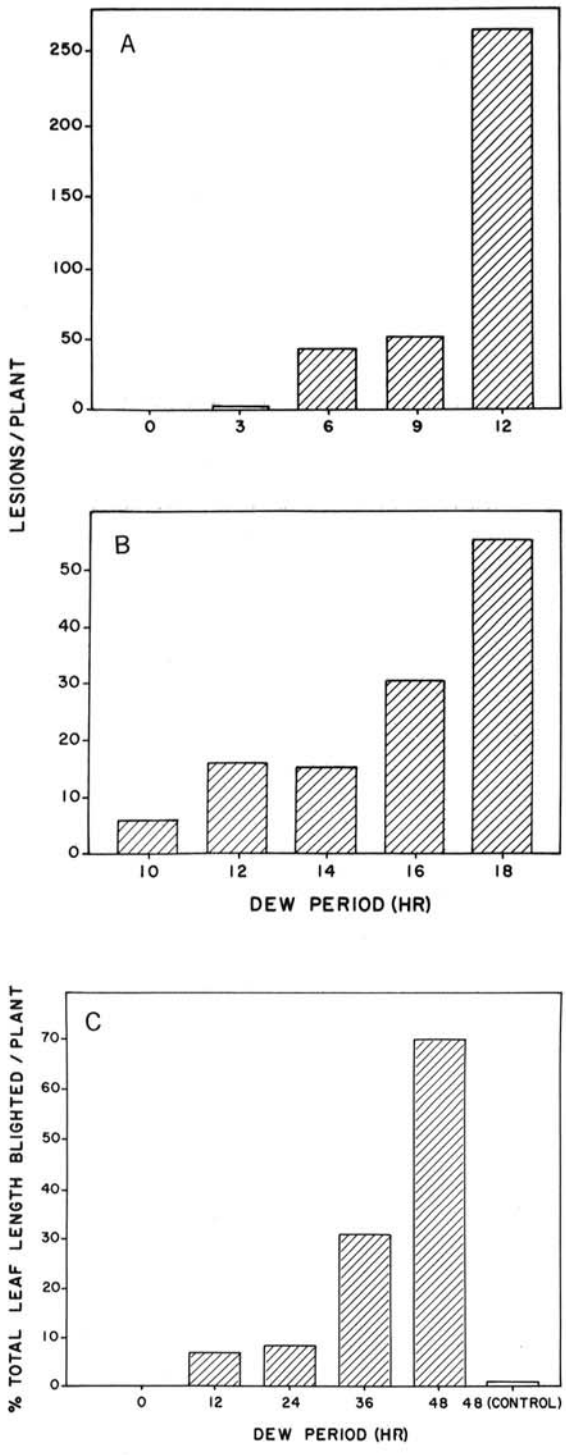

Fig. 2-(A to C). Relation between dew period, lesion production, and leaf blighting on onion plants exposed to different dew periods following inoculation with *Botrytis squamosa* in three different experiments: **A)** Dew period up to 12 hr, 10^4 conidia in 5 ml per plant (cultivar Elite). **B)** Dew period up to 18 hr 6×10^4 conidia in 3 ml per plant (cultivar Elite). **C)** Dew period up to 48 hr, 1.25×10^5 conidia in 2.5 ml per plant (cultivar Spartan Banner).

single-drop spore suspensions of isolate 64a were pipetted onto glass slides in sterile petri dishes which were lined with moistened filter paper and equilibrated to the appropriate temperatures. Spores were incubated for 12 or 24 hr at 11 different temperatures ranging from 3 to 33 C at 3-C intervals. Spores were treated with 2% formaldehyde and stained with cotton blue in lactophenol prior to observation for germination. Spores were counted as germinated if the germ tube was as long as the diameter of the spore.

Temperature and lesion production.—The relationship between temperature and lesion production was studied during continuous leaf wetness. Onion seeds (cultivar Elite) were planted at 2-day intervals in lots of 16 pots (20-cm diameter) starting 2 mo before the first temperature study. Five transfers of isolate 64a were made at 2-day intervals commencing 2 wk before the first temperature study. This procedure provided plants of equal age and inocula of equal age for each study. In each temperature study, six plants were placed in the dew chamber at 1100 hours. By 1700 hours, the time of inoculation, dew formation had occurred on the leaves (Fig. 1-B). Plants were inoculated with a suspension of 8×10^4 conidia in 4 ml per plant as previously described. After 40 hr of incubation, the lesions were counted on a 20-cm section measured from the leaf tip of the fourth leaf from the center for each of the six plants. Lesion sizes were measured at 4, 8, 12, and 16 cm from the leaf tip of these same leaves. This procedure was used in 24 different temperature studies in the range 7-31 C.

RESULTS

Dew and pathogenesis.—Lesions did not develop on leaves which were maintained in an atmosphere of 92% RH for 48 hr, when spores of isolate 64a were brushed on dry (Fig. 1-D). However, lesions did develop on leaves which were exposed to a wetness period of 48 hr after the brushing on of spores of isolate 64a (Fig. 1-F).

Dew period and lesion production.—Numbers of lesions increased with longer dew periods in both studies (Fig. 2-A, B). In the first study involving dew periods up to 12 hr, 6 hr was the minimum dew period required for significant lesion formation and 12 hr gave the largest number of lesions (Fig. 2-A). In a second study with dew periods up to 18 hr, most lesions per plant were produced after 18 hr of dew (Fig. 2-B).

In the 12-hr dew period study, leaf position did not contribute significantly to variation in numbers of lesions, whereas in the 18-hr study, numbers of lesions increased progressively with increased age of leaves proceeding outward from the center of the plant.

Dew period and blighting.—Leaf blighting of inoculated plants from sprouted bulbs increased with time in the dew chamber up to 48 hr, the longest time tested (Fig. 1-C, 2-C). Examination of the data by the analysis of covariance indicated that hours of dew contributed significantly ($P = 0.05$) to variation in the amount of blighting.

Dew period, blighting, lesion production, and leaf position.—The amount of blighting increased progressively with leaf age from the youngest (position 1) to oldest (position 5) leaf, regardless of dew period (Fig. 3-A). However, the longer the dew period the greater the

amount of blighting at all leaf positions. The controls showed the same relationship between amount of blighting and leaf position, but blighting was considerably less than for the inoculated plants. The analysis of covariance indicated that variation in the amount of blighting could be attributed to leaf position, hours of dew, and numbers of lesions ($P = 0.05$).

The relationship between lesion production and leaf position also was investigated in this experiment. For 36-, 60-, and 84-hr dew periods, the numbers of lesions increased with leaf age (Fig. 3-B). Leaf position 1 (youngest) generally had fewer lesions than leaf positions 2, 3, 4, or 5 (oldest).

Temperature and mycelial growth.—Isolates 62-10, 64a, and 69-13 had cardinal temperatures for growth near 9, 24, and 31 C, respectively (Fig. 4-A, B). Graphs of colony radius versus temperature for the four isolates resulted in curves that were similar in shape and skewed toward lower temperatures (Fig. 4-A). Isolate 66-6 exhibited poor growth at all temperatures. Graphs of colony weight versus temperature for the four isolates were not similar in shape although curves for three of the isolates were skewed toward lower temperatures (Fig. 4-B).

Temperature and conidial germination.—The cardinal temperatures for spore germination for isolate 64a were near 12, 15, and 27 C (Fig. 4-C). The curves resulting from plotting percentage germination after 12 and 24 hr incubation versus temperature were skewed toward higher temperatures (Fig. 4-C). Conidia germinated over a narrower temperature range and had a lower optimum than that determined for mycelial growth.

Temperature and lesion production.—Lesions

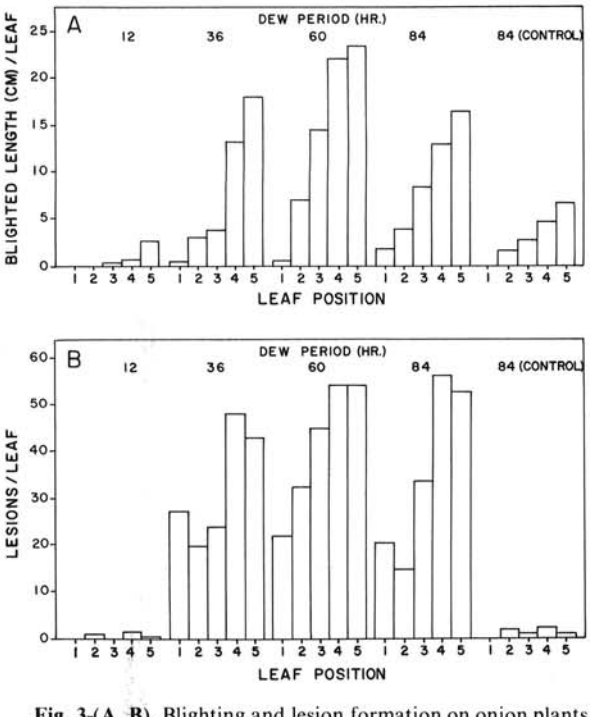

Fig. 3-(A, B). Blighting and lesion formation on onion plants (cultivar Elite) exposed to different dew periods following inoculation with *Botrytis squamosa* at 8×10^4 conidia in 4 ml per plant. The noninoculated control was exposed to 84 hr of dew. Leaf position (1-5) was numbered from the center leaf (youngest) to the next from center leaf and so on to the fifth leaf (oldest of those measured). Bars represent the average value for three replicates. **A)** Relation between dew period, leaf position, and blighting. **B)** Relation between dew period, leaf position, and number of lesions.

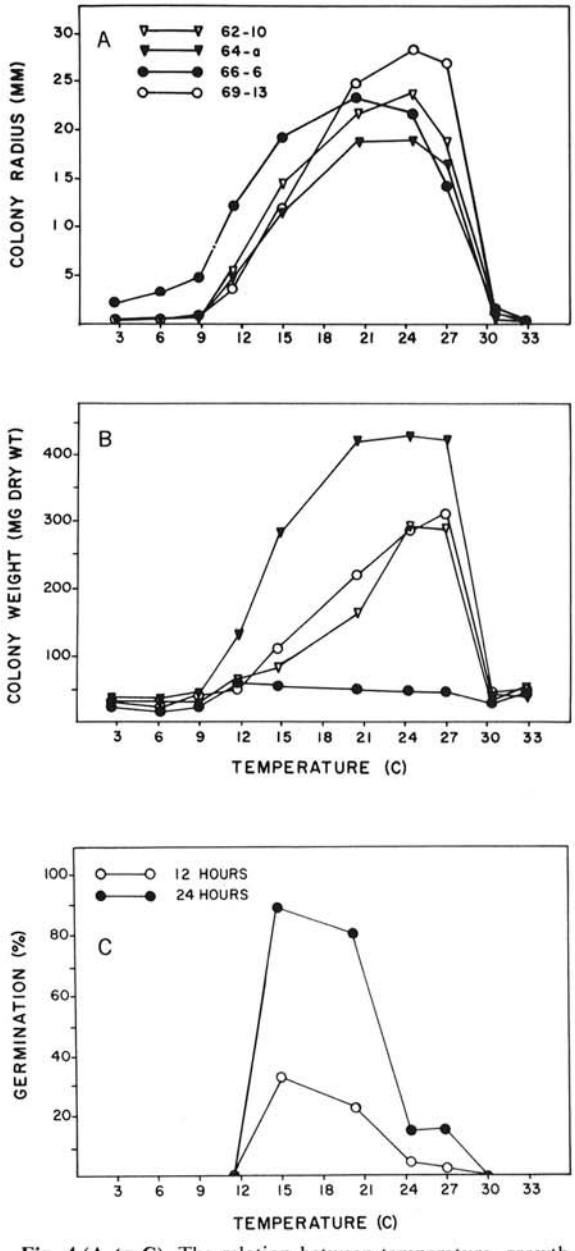

Fig. 4-(A to C). The relation between temperature, growth, and germination of *Botrytis squamosa* conidia. Wild-type isolates 62-10 and 69-13 from Orange County, N.Y. and 66-6 from Long Island, N.Y. Mutant isolate 64a from R. R. Bergquist. **A)** Colony radius (mm) measured after 3 days growth on PDA; six replicates per temperature treatment. **B)** Colony weight (mg dry wt) measured after 5 days growth in PDB; four replicates per temperature treatment. **C)** Germination of conidia of mutant isolate 64a after 12 and 24 hr.

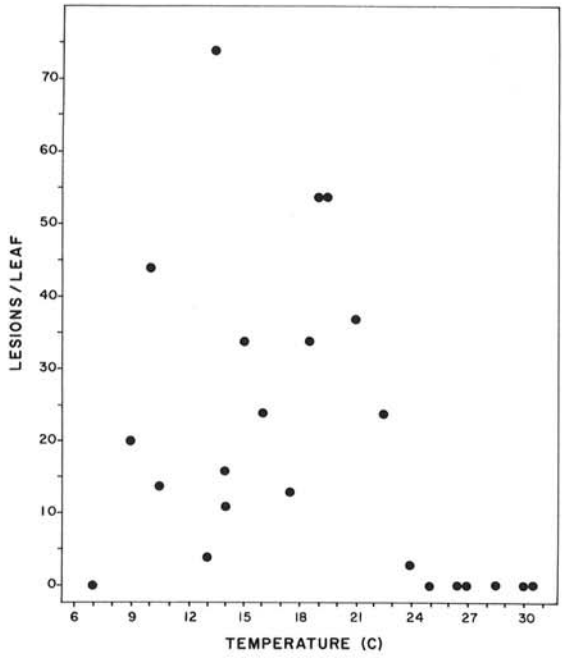

Fig. 5. Relation between temperature and lesion development 40 hr after inoculation with *Botrytis squamosa* at 8×10^4 conidia in 4 ml per plant on onions (cultivar Elite) under constant dew. Lesions were counted on 20-cm length (measured from the leaf tip) of the fourth leaf (counted from the center leaf) of six plants per temperature treatment.

developed between 9 and 23 C (Fig. 5). Because of variation among the temperature studies, the shape of the temperature-response curve or the optimum temperature for lesion development could not be determined. Lesions that developed at the various temperatures were similar in size (approximately 0.6×1.0 mm) and shape.

DISCUSSION

Increased numbers of lesions coincided with an increase in the duration of the postinoculation dew period. Longer dew periods allow more time for conidial germination and penetration to take place.

Blighting also increased as the duration of dew was increased. In the field, extended wet periods probably play an important role in pathogenesis by *B. squamosa*. During relatively dry periods, when leaves are wet only from nightly dew periods (about 12 hr), disease development is restricted to lesion production with blighting being minimal. However, during long wet periods, disease development is enhanced, with numbers of lesions and blighting both greatly increased.

Our data and those from previous studies (6, 7, 8) indicate that lesion production and blighting are influenced by leaf position. The younger leaves were less susceptible than the older leaves. This could be related to leaf maturity and the ability of a younger, physiologically more active leaf to resist disease. Another explanation for this difference, which we did not investigate, is that "spore catch" is not similar on all leaf ages because of differences in leaf surfaces (e.g., wax content).

Optimum temperature for germination of *B. squamosa* conidia (approximately 15 C) was lower than for mycelial growth (approximately 24 C). In nature, presumably, conidial germination occurs at night when lower temperatures and dew are present, and ingress and infection occur the following morning and daytime when higher temperatures are present.

The temperature range (9 to 23 C) for lesion development in the controlled-environment studies was different from the range (9 to 30 C) for mycelial growth. This difference is explained by the fact that plants in the lesion/temperature study were exposed to a constant temperature from inoculation to when the lesions were counted. Since germination is very poor at 24 C and higher, it would be expected that lesion production at temperatures above 24 C would be neglibible. It is probable that lesions would have developed at temperatures higher than 23 C had the initial temperatures been optimal for germination, and then raised for lesion development.

In the conidial germination and growth experiments at different temperatures, mutant isolate 64a responded similarly to isolates 62-10 and 69-13. Isolate 66-6 responded differently from the other three isolates. Because 64a sporulates more readily than the other isolates (2) and is equally pathogenic, it is very useful for laboratory studies in which a constant and easily obtained supply of conidia are required.

LITERATURE CITED

1. BERGQUIST, R. R., R. K. HORST, and J. W. LORBEER. 1972. Influence of polychromatic light, carbohydrate source, and pH on conidiation of Botryotinia squamosa. Phytopathology 62:889-895.
2. BERGQUIST, R. R., and J. W. LORBEER. 1973. Genetics of variation in Botryotinia squamosa. Mycologia 65:36-47.
3. DIXON, W. J. (ed.). 1970. BMD Biomedical Computer Programs, Second Edition. University of California Publications in Automatic Computation No. 2. Univ. Calif. Press, Berkeley and Los Angeles. 600 p.
4. HANCOCK, J. G., and J. W. LORBEER. 1963. Pathogenesis of Botrytis cinerea, B. squamosa, and B. allii on onion leaves. Phytopathology 53:669-673.
5. HICKMAN, C. J., and D. ASHWORTH. 1943. The occurrence of Botrytis spp. on onion leaves with special reference to B. squamosa. Trans. Br. Mycol. Soc. 26:153-157.
6. MC LEAN, D. M., and B. SLEETH. 1959. Tip and leaf blight of onions in the lower Rio Grande Valley. J. Rio Grande Valley Hortic. Soc. 13:152-154.
7. PAGE, O. T. 1953. Botrytis spot of onion leaves in Ontario. Plant Dis. Rep. 37:513-514.
8. PAGE, O. T. 1955. Botrytis leaf spot on onions and its control. Can. J. Agric. Sci. 35:358-365.
9. SEARLE, S. R. 1971. Linear models. John Wiley and Sons, New York. 576 p.
10. SEGALL, R. H. 1953. Onion blast or leaf spotting caused by species of Botrytis. Phytopathology 43:483 (Abstr.).
11. SEGALL, R. H., and A. G. NEWHALL. 1960. Onion blast or leaf spotting caused by species of Botrytis. Phytopathology 50:76-82.
12. SHOEMAKER, P. B., and J. W. LORBEER. 1971. The role of dew and temperature in the epidemiology of Botrytis leaf blight of onion. Phytopathology 61:910 (Abstr.).
13. VIENNOT-BOURGIN, G. 1952. Sur la présence en France de Botrytis squamosa, parasite de l'oignon. Rev. Pathol. Veg. Entomol. Agric. 31:82-98.