Influence of Dew Period and Temperature on Infection of Onion Leaves by Dry Conidia of *Botrytis squamosa*

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ABSTRACT

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A dry spore inoculation technique was used to examine the influence of dew period and temperature on infection of onion leaves by *Botrytis squamosa*. A linear relationship was found between mass of dry conidia used as inoculum and lesion numbers. Dry spores applied to leaf surfaces survived 2 days with no reduction in their potential to induce lesions, but lesion numbers declined when inoculated plants were held in a growth chamber at 60% relative humidity (RH) for more than 2 days prior to incubation in a dew chamber. Lesion production was optimal at 20 C, lower at 15 C, and greatly reduced at 25 C. Lesions were initiated after 6 hr of dew, and numbers of lesions increased sigmoidially through 32 hr of dew. On leaf surfaces, the percentage of conidia that germinated, and the proportions of germinated conidia that subsequently formed appressoria, formed infection hyphae, and resulted in lesions were greater at 20 C than at 25 C. Numbers of lesions averaging 62 ± 8% of dry conidia deposited on leaves resulted after 24 hr of continuous dew at 20 C. Hyphal development within lesions increased with time through 6 days of continuous dew at 20 C, but was restricted under conditions of no dew and moderate (60 ± 10%) RH.

Additional key words: *Allium cepa*, Botrytis leaf blight, epidemiology.

On onion (*Allium cepa* L.) leaves, *Botrytis squamosa* Walker induces 1- to 5-mm-diameter lesions that, under prolonged moist conditions, may expand and coalesce to give leaf blighting (2,4,8,10). Leaf wetness and temperature are considered important parameters in the infection of onion by *B. squamosa* (7–11). Although the role of leaf wetness and temperature in leaf blight development has been examined by using aqueous conidial suspensions as inoculum (7,8,10,11), the germination of "dry" conidia on leaf surfaces, and subsequent penetration and infection, as influenced by duration of dew at various temperatures, is not clearly understood. Conidia are released during the morning hours when relative humidity is dropping, are transported by air currents, and deposited on the onion leaf in the absence of free water, and germinate and infect after the leaves have been wet by dew or rain (5,6,9,10).

Germination of conidia of *B. squamosa* has been examined in water on glass slides and leaf surfaces. Shoemaker and Lorbeer (8) reported optimal conidial germination on glass slides at 15 C with no germination above 27 C. Swanton (10) reported optimal germination at 20 or 28 C with reduced germination at 33 C. McDonald (7) reported optimal germination at 24 C. Histology of conidial germination on leaf surfaces and subsequent infection at 21 C was studied by Clark and Lorbeer (2), but temperature influences on germination were not determined. Rate of germ tube elongation was greater in water than in nutrient solution on leaf surfaces (1).

Several studies have addressed the effect of temperature and/or leaf wetness on infection of onion by *B. squamosa*. The optimum temperature for leaf blight development was reported to be 18 C (7,11) and 20 C (8,10). Lesion development has been reported to be reduced at temperatures above 24 C (7,8,11). At 9–25 C, lesion numbers were reported to increase with increasing temperature and leaf wetness duration (7,8,10,11). Conflicting reports have been published on optimum leaf wetness periods for maximum infection. Using conidial suspensions, minimal wetness durations for subsequent development of lesions at 18–20 C have been reported to be 12 (11), 9 (7), 6 (8), or 5 hr (10) while maximal lesions were reported to occur after 12 (7,8), 18 (8), 24 (11), or 48 hr (10).

Preliminary experiments gave highly variable numbers of lesions when plants were inoculated with aqueous conidial suspensions. Much more consistent results were obtained by applying the conidia in a dry state in a settling tower, so we used the dry conidia inoculation technique throughout these experiments.

The objectives of this study were: to determine the effect of length of dew period at various temperatures on conidial germination and

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subsequent infection and lesion production in onion leaves exposed to a standardized number of conidia under controlled environmental conditions, with the conidia applied in a dry state similar to that occurring in nature; and to determine the effect of extended dew duration on postinfection hyphal development within lesions.

MATERIALS AND METHODS

Inoculum production. Isolate BSS-4 of *B. squamosa* was used in all experiments because it was highly virulent and it sporulated somewhat more prolifically than other isolates. The fungus was grown on potato-dextrose agar (PDA) for 7–12 days, transferred to sterilized muck soil in test tubes, incubated 7–12 days at room temperature, and then stored at 5 C. For conidial production, infested muck particles were placed on autoclaved (85 min at 1.02 kPa [20 psi]) onion leaves placed aseptically on the surface of 1.5% water agar in 9-cm-diameter petri plates. The plates were incubated at 20 C under a 16 hr fluorescent light photoperiod for 5–7 days to induce conidial formation.

Inoculation procedure. In all experiments we used 1-mo-old onion plants (cultivars Spartan Banner, Yellow Sweet Spanish, or Spartan Sleeper) sprouted from bulbs. Conidia of *B. squamosa* were collected from aerial conidiophores on autoclaved onion leaves with a Pasteur pipet connected to a water aspirator operated at low suction. Spore numbers were estimated by weight. For inoculation, plants were positioned within a 61 cm (diameter) × 77 cm (deep) cylindrical settling tower and dry conidia were dispensed near the top of the chamber by directing a low velocity stream of air from a pipet tip over the conidia on a piece of weighing paper. A cover was positioned over the top of the cylinder for about 5 min to reduce air currents and allow the spores to settle on the plants. The plants were then incubated in a commercial dew chamber (Percival Mfg. Co., Boone, IA 50036) for the appropriate dew periods. Lesions were always evenly distributed on leaves using this technique.

Influence of inoculum density on lesion numbers. To determine the relationship between inoculum density and lesion numbers, groups of six replicate onion plants were each inoculated with 0.5, 1.0, 1.5, 2.0, or 2.5 mg of dry conidia and placed in a dew chamber at 20 C for 24 hr. Lesion numbers were counted at the end of the incubation period.

Influence of dew period and temperature on lesion production. Groups of 18 onion plants were inoculated with dry conidia and placed in the dew chamber at 15, 20, or 25 C. Six randomly preselected plants were removed from the dew chamber after each period of 4, 8, 12, 16, 24, or 32 hr of continuous dew, respectively, and were then moved to a growth chamber set at the same temperature as the dew chamber, with 60 ± 10% relative humidity (RH) and a 16-hr photoperiod. Lesions were counted on each plant after the variable times in the dew chamber and growth chamber totaled 48 hr. The experiment was repeated three times.

Influence of temperature on spore germination and infection. Four onion plants were inoculated in the settling tower with 2 mg dry conidia and placed in a dew chamber for 24 hr at 20 or 25 C. The third or fourth youngest leaf on each plant was used, since lesion size distribution was more uniform on these leaves. Four 1-cm^2 leaf tissue pieces were removed from each of four replicate leaves on four separate plants, fixed in FAA, stained with cotton blue (6), and examined with light microscopy. Conidia that were washed off leaves during fixation were collected on a 13-mm-diameter filter membrane (Millipore Filter Corp., Bedford, MA 01730), stained with cotton blue, and counted with a light microscope.

Numbers of conidia on leaves that had formed appressoria were counted, as well as spores that had formed both appressoria and infection hyphae. Appressorial counts were based on swollen germ tube tips, and infection hyphal counts were based on the observation of infection hyphae within lesions. The experiment was repeated three times.

Influence of dew period on infection hyphal development. Sixteen onion plants were inoculated and placed in the dew chamber for 2, 4, or 6 days of continuous dew at 20 C with a 12-hr photoperiod. In addition, four plants were given a 2-day dew period at 20 C, then incubated in a growth chamber maintained at 20 C, 60 ± 10% RH and a 12-hr photoperiod for 4 days. Lesions were randomly removed from the plants, fixed in FAA, cleared in boiling 70% ethanol, and stained in 1% aqueous trypan blue. Lengths of infection hyphae were measured with an ocular micrometer for a minimum of 50 lesions. The experiment was repeated three times.

RESULTS

Influence of inoculum density on lesion numbers. Lesion numbers increased linearly when from 0.5 to 2.5 mg of conidia per 0.3 m^2 area were applied to onion plants subsequently incubated for 24 hr in a dew chamber at 20 C (Fig. 1). To determine the number of conidia per unit weight, different weights of conidia were suspended in 5 or 10 ml of distilled water and samples were counted with a hemacytometer. Amounts of conidia weighing 0.5, 1.0, 1.5, 2.0, and 2.5 mg were determined to contain ~2.5, 4.9, 7.4, 10.0, and 12.5 × 10^5 conidia, respectively. These values represented the conidial numbers falling on the floor area in the settling tower.

Longevity of spores on onion leaves. To determine the longevity of spores on onion leaves following inoculation, two groups of 21 onion plants were inoculated with 4 mg conidia and placed in a growth chamber at 20 C. After 0, 1, 2, 3, 4, 6, or 8 days, six replicate plants were removed at the appropriate time from the growth chamber and incubated with continuous dew for 36 hr, then returned to the growth chamber. Numbers of lesions per plant were counted 24 hr after removal from the dew chamber.

Under conditions of 60 ± 10% RH and 20 C, numbers of lesions remained relatively constant on leaves held for up to 2 days after inoculation in the growth chamber followed by a 36-hr dew period (Fig. 2). There were ~425–450 lesions per plant with a 0–2 day interval between inoculation and exposure to dew. With a 3–4 day interval this declined to ~245 lesions per plant, and with a 6- or 8-day interval this further declined to ~130 per plant, reflecting a 71% loss in infectivity of conidia by day 6.

Influence of dew period and temperature on infection. Lesions were produced after 8, but not after 4, hr of continuous dew at 20 C. The number of lesions per plant increased sigmoidally with increasing dew duration, with little increase in lesion numbers after

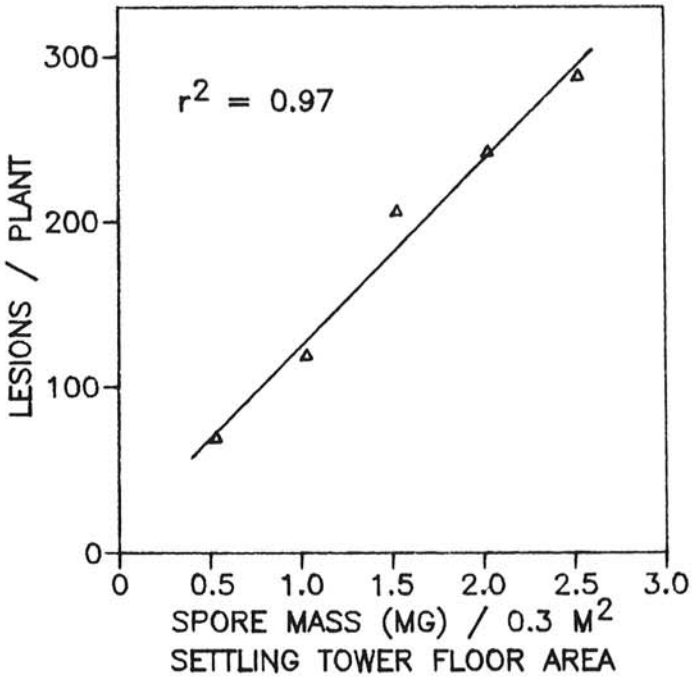

Fig. 1. Relationship between mass of conidial inoculum of *Botrytis squamosa* and lesion numbers on 1-mo-old onion plants after incubation in a dew chamber at 20 C for 24 hr.

24 hr of dew (Fig. 3). Total numbers of lesions produced at 20 or 15 C were approximately equal on plants incubated with constant dew for 32 hr, although the rate of increase in lesion production at incremental dew periods was slower at 15 than 20 C. Lesion production was severely curtailed at 25 C and did not increase after 12 hr of continuous dew.

To determine the minimum dew period required for infection, six onion plants were inoculated with dry conidia and placed in the dew chamber at 20 C. After 4, 5, 6, or 7 hr, plants were transferred to a growth chamber set at 20 C and 60 ± 10% RH. Lesions were counted after an additional 24 hr in the growth chamber. No lesions were observed on plants given 4- or 5-hr dew periods. After 6- or 7-hr of dew, 4.2 ± 4 and 29 ± 18 lesions per plant, respectively, were observed.

Influence of temperature on spore germination and infection. At 20 C, 78 ± 10% of the conidia germinated on leaves while 62 ± 8% induced lesions. At 25 C, 55 ± 5% of the spores germinated while only 27 ± 10% induced lesions. At 20 C, 61 ± 8% of the spores formed appressoria and 36 ± 6% formed infection hyphae, compared with 37 ± 1% forming appressoria and 11 ± 6% forming infection hyphae at 25 C.

Influence of dew period on development of infection hyphae. Lengths of infection hyphae within lesions after 2 days in the dew chamber were less than 125 μm (Table 1). After 4 days ~30% of the hyphae extended beyond 125 μm with the greatest number falling into the 125–250 μm category. After 6 days of continuous dew ~35% of the hyphae extended beyond 125 μm, again with the greatest number in the 125–250 μm category. Infection hyphae in lesions on plants treated 2 days with dew and 4 days without dew in the growth chamber remained restricted, with only 4% reaching the 125–250 μm category, and none falling into the longer categories.

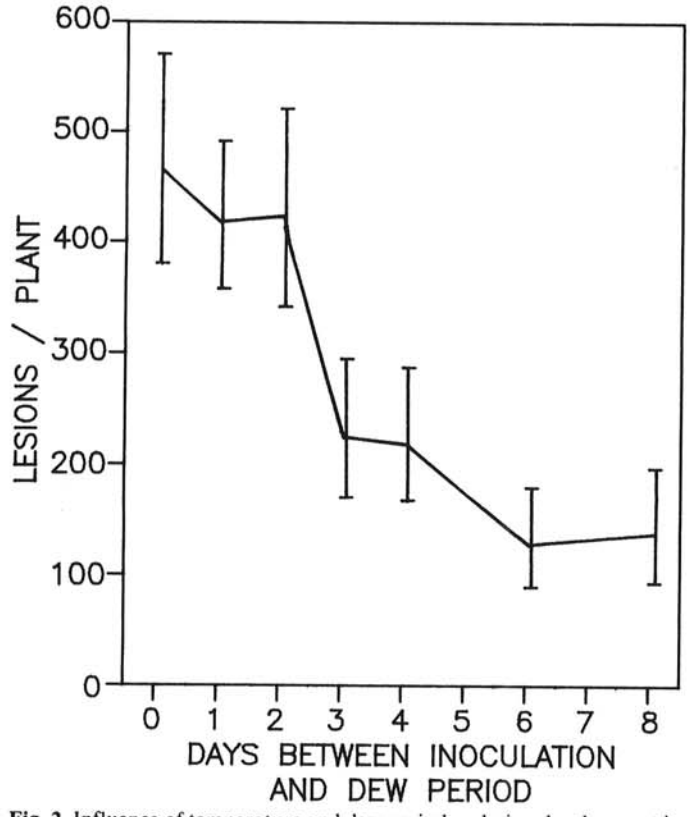

Fig. 2. Influence of temperature and dew period on lesion development in 1-mo-old onion plants following inoculation in a settling tower, with 2 mg dry conidia of *Botrytis squamosa*. Mean values were derived from three replicate experiments; LSD ($P = 0.05$) between 8 and 32 hr was 41.

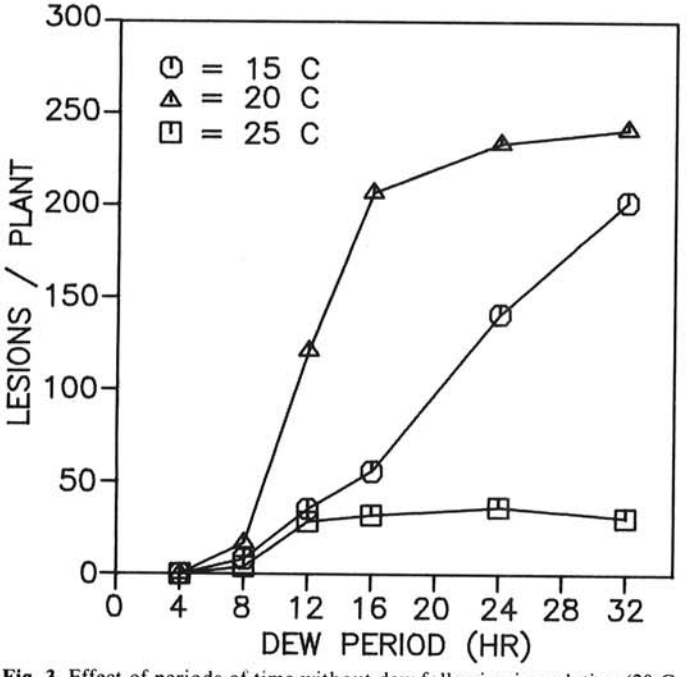

Fig. 3. Effect of periods of time without dew following inoculation (20 C and 60% RH) prior to exposure to 24 hr of continuous dew at 20 C on numbers of lesions per plant. Plants were inoculated in a settling tower with 4 mg of dry conidia of *Botrytis squamosa*.

DISCUSSION

Previous dew period studies employed aqueous conidial suspensions as inoculum. However, under field conditions conidia of *B. squamosa* are disseminated by wind and deposited on leaves as dry spores (4,10). Examining environmental influences on spores applied in a dry condition to leaves, followed by leaf wetness as dew, should give us additional understanding of the infection process as it occurs in the field.

Most conidia of *B. squamosa* are released between 0800 and 1300 hours (5,6), becoming windborne until deposited on leaves (5,9). Results of this study suggest that *B. squamosa* conidia can survive on leaf surfaces for 2–3 days after deposition without a significant loss of viability. Survival could be shorter on leaves exposed to full sunlight. Similarly, Shoemaker and Lorbeer (8) reported that conidia brushed onto leaf surfaces of plants in growth chambers survived 2 days at 92% RH. A survival study of conidia in soil (3) also suggested relatively short-term survival of conidia.

Lesion production after a minimum 6-hr dew period was consistent with the observations of Shoemaker and Lorbeer (8), who, using aqueous conidial suspensions, reported a 6-hr minimum period of leaf wetness for lesion development. We observed that relatively few lesions were produced on inoculated plants exposed to only 6 hr of dew at 20 C, and a sharp increase in lesion numbers occurred with 12 or more hr of dew at 20 C, which also agreed with Shoemaker and Lorbeer (8).

The slower rate of lesion production at 15 C compared with 20 C could be significant in understanding lesion production under field

TABLE 1. Percentage of infection hyphae of *Botrytis squamosa* falling within ranges of lengths in lesions on onion leaves after various dew periods at 20 C

Infection hyphae lengths (μm)	Length of dew period (days)			Dew 2 days and no dew 4 days
	2[a]	4[a]	6[a]	
1–125	100[b]	72 ± 15	65 ± 14	96 ± 6
125–250	0	13 ± 8	17 ± 9	4 ± 6
250–375	0	7 ± 4	5 ± 5	0
375–500	0	3 ± 3	4 ± 1	0
>500	0	5 ± 1	9 ± 3	0

[a] Lesions were examined immediately after the end of the indicated dew period.

[b] Based on three replicate experiments with each experimental run including a minimum of 50 lesion observations.

conditions. Dew periods shorter than 16 hr could result in significantly fewer lesions at 15 than at 20 C (Fig. 3), and temperatures of 25 C or greater will also result in fewer lesions (Fig. 3) (7,8).

Shoemaker and Lorbeer (8) reported that spore germination on glass slides declined from 80% at 21 C to 20% at 24 C, while McDonald (7) and Swanton (10) reported optimal germination at 24 C on glass slides. We observd a sizeable reduction in spore germination on leaves at 25 C compared to 20 C. The reduced spore germination and proportionally greater reduction in infection hyphae could account for the reduced lesion production noted at 25 C.

Extent of pathogen development within lesions was dependent, at least in part, upon length of dew period. After 4 days of continuous dew, some lesions were expanding and initiating the leaf blight phase of the disease (Table 1), as reported by Clark and Lorbeer (2). Observations of hyphal length revealed that hyphae continued to grow in 5–15% of the original lesions and were responsible for the rapid and destructive leaf blight phase of the disease. The reason for some lesions expanding and others remaining static in size is not yet understood, nor is the effect of extended dew periods on expansion of a larger proportion of lesions understood. Understanding these aspects of disease development could be important epidemiologically.

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