Influence of Interruptions of Dew Period on Numbers of Lesions Produced on Onion by *Botrytis squamosa*

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ABSTRACT

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Onion plants inoculated with dry conidia of *Botrytis squamosa* and placed in a dew chamber for 6 hr, followed by a dry period of variable (0.3–24 hr) duration, and returned to the dew chamber for the remainder of the incubation period, had progressively fewer lesions as duration of the dry interruption period increased. Lesion numbers also tended to decline as relative humidity decreased from 90 to 30% during a relatively short 20-min dew interruption period. Plants similarly inoculated showed increasing spore germination, appressorium formation, numbers of lesions, and visible infection hyphae as dew periods were increased from 2 to 24 hr. Plants were inoculated, given an initial dew period of from 2 to 12 hr, a 2-hr dry period, and a subsequent dew period of 12–22 hr for a total incubation period of 26 hr. Since these plants received 24 hr of dew in a 26 hr period, the only variable was the timing of the 2-hr dew interruption. Conidial germination was near 100% after 26 hr, regardless of timing of the dry period. However, appressorium formation and numbers of lesions were both reduced more by dew interruptions occurring after 6 hr of initial dew than by interruptions occurring after 2, 4, 8, 10, or 12 hr of initial dew, presumably because germinating conidia were most vulnerable to drying at this time. Numbers of visible infection hyphae were lowest when dry periods occurred after a 6- or 8-hr initial dew period.

Botrytis leaf blight, incited by *Botrytis squamosa* Walker, is a leaf-spotting and blighting disease which is especially severe under prolonged moist conditions at temperatures of 15–24 C (5,7,9,10). Studies relating dew period and temperature to leaf blight development indicated that greatest numbers of lesions developed at 18–20 C and that lesion numbers increased with increasing leaf wetness durations of up to 48 hr (1,6,8,10,11). Leaf lesions developed within 24 hr after inoculation and incubation under constant leaf wetness at 15–20 C (1). Most lesions remain the same size (1–3 × 2–4 mm), although a small proportion of them may continue to increase in size and lead to blighting if leaf wetness is prolonged (1,3).

Experiments relating interruptions of postinoculation leaf wetness to subsequent lesion numbers produced by *B. squamosa* have not clearly established the relationships of timing or duration of leaf wetness interruptions to lesion development, and no studies have related these factors to pathogen development either upon or within leaves. McDonald (6) examined the influence of wet-dry-wet periods of 4-4-20, 4-8-20, 8-4-16, 0-8-24, and 0-0-24 hr respectively, on lesion development, and found fewer lesions on plants given a postinoculation dry period compared with those provided continuous leaf wetness. Swanton (10) placed inoculated plants under 2- or 8-hr initial wetness periods, 2-, 6-, 9-, or 12-hr dry periods, and a 16-hr resumed wetness period. Increasing lengths of dry periods resulted in decreasing numbers of lesions. However, he failed to include inoculated and uninoculated control plants receiving continuous leaf wetness. Dzikowski (4) examined the influence of 1- or 4-hr dry periods following various initial wetness periods on disease incited by *B. squamosa*. He observed a lower percent diseased leaf area when dew was interrupted (1 or 4 hr) following 5 hr of initial leaf wetness than following 2 hr of initial leaf wetness. However, treatment responses were not quantified in terms of lesion numbers and it is not clear how treatment differences, expressed in terms of percent leaf area diseased, were obtained. All of these investigators used aqueous spore suspensions for inoculations, which we found to give less consistent results than the dry spore inoculation technique (1).

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Under field conditions, conidia are subjected to cyclic wetting and drying, often more than once in a 24-hr period, at least in Michigan (*unpublished*). Understanding the influence of these factors on infection of onion by *B. squamosa* could be epidemiologically important.

The objectives of this study were to determine the influence of duration and timing of dry periods interrupting dew periods and of relative humidity (RH) during dry interruptions, on numbers of lesions produced on onion leaves by *B. squamosa*, and to determine the effect of leaf wetness duration and timing of dew period interruptions on conidial germination, appressorium formation, lesion formation, and numbers of visible infection hyphae.

MATERIALS AND METHODS

Botrytis squamosa was grown and spores were collected as previously described (1). One-month-old onion plants (*Allium cepa* L. 'Spartan Banner', 'Granada', or 'Yellow Sweet Spanish') sprouted from bulbs were used. For inoculation, plants were positioned within a cylindrical settling tower 61 cm in diam and 77 cm deep. Dry conidia (2.5 mg, or about 1.25×10^6 conidia) were dispersed near the top of the tower by gently blowing air from a pipet tip over the conidia on a piece of weighing paper. During inoculation, the plants were rotated on a turntable in the chamber at 5–6 rpm (1). After the conidia were dispersed, a cover was placed over the top of the tower for about 5 min to reduce external air currents and allow the spores to settle on leaf surfaces. Because of the limited size of the settling tower, plants within an experiment were randomly assigned to two groups, and each group was inoculated separately. The chamber was vacuumed between inoculations. After inoculation, both groups of plants were placed in a commercial dew chamber (model 1-35 DL; Percival Mfg. Co., Boone, IA), in which visible dew was produced on leaves in less than 1 hr. Lesion numbers on the two groups of plants within an experiment were not significantly different.

Influence of leaf wetness duration prior to dry periods. Forty-two onion plants were inoculated as described above and placed in the dew chamber at 20 C for 2, 4, 6, 8, 10, or 12 hr were moved to a growth chamber (20 C, 65 ± 10% RH) for a 2-hr dry period, and were finally returned to the dew chamber for the remainder of the 24-hr incubation period. Control plants remained in the dew chamber for 24 hr. Lesions visible to the unaided eye on each plant were counted after an additional 6 hr in the growth chamber.

Influence of dry period duration on lesion production. Experiments were initiated to examine the influence of brief (0.3–1.7 hr) and extended (2–24 hr) postinoculation dry periods following initial dew periods on numbers of lesions produced by *B. squamosa*. Thirty-six onion plants were inoculated, incubated in the dew chamber at 20 C for 6 hr, then transferred to a growth chamber at 20 C. Dew formed on leaves in the dew chamber as uniform fine droplets in less than 1 hr, and evaporated in less than 5 min after placement in the growth chamber. After a 0.3-, 0.7-, 1.0-, 1.3-, or 1.7-hr dry period in the growth chamber, six replicate plants for each dry period were returned to the dew chamber for the remainder of the 24-hr incubation period. Plants were then placed in a growth chamber for an additional 6 hr, then lesions were counted. Uninoculated controls remained in the dew chamber continuously for 24 hr, then were held in a growth chamber for 6 hr prior to lesion assessment. The experiment was conducted twice.

To examine the effect of longer postinoculation dry periods, 24 onion plants were inoculated, incubated in the dew chamber at 20 C for 6 hr, and then were transferred to a growth chamber at 20 C and 65 ± 10% RH. After 4, 8, or 24 hr in the growth chamber, six plants from each dry period treatment group were returned to the dew chamber for an additional 24 hr. Uninoculated controls remained in the chamber for 54 hr. Experiments were conducted twice.

Influence of relative humidity during dry periods. Following inoculation and 6 hr in the dew chamber at 20 C, six plants were removed and placed in a growth chamber at 30, 60, or 90% ± 10% RH for 20 min and then returned to the dew chamber. Six control plants remained continuously in the dew chamber for 24 hr. Humidity and temperature were monitored with a recording hygrothermograph calibrated and checked before and after the experiment against readings obtained with a sling psychrometer. The experiment was conducted three times.

Influence of dew period duration and timing of dry periods on spore germination and infection. Twenty-eight onion plants were inoculated, then placed in the dew chamber at 20 C for 2, 4, 6, 8, 10, 12, or 24 hr. The third or fourth youngest leaves of four plants were used for sampling because lesions produced on these leaves were the most uniform in size. Four 1 cm^2 leaf tissue pieces were removed from each leaf, fixed in formalin-50% ethanol-glacial acetic acid (1:18:1, v/v), stained with cotton blue in lactic acid (28 mg of aniline blue, 20 ml of distilled water, 10 ml of glycerol, and 10 ml of 85% lactic acid), mounted on slides, and examined under the light microscope. Germinated and ungerminated conidia, and numbers of appressoria, lesions, and visible infection hyphae were counted and expressed as the percentage of the total number of conidia counted on a leaf sample. Conidia that were washed from the leaves during fixation were collected by passing the fixative through 13-mm-diameter membrane filters (0.33 μm pore diameter; Millipore Filter Corporation, Bedford, MA). These were mounted on slides, stained, and the germinated and ungerminated conidia were counted as above. The conidia were included in the totals for each leaf sample.

A similar experiment was conducted, except that a 2-hr dry interruption of dew was inserted after 2, 4, 6, 8, 10, or 12 hr of initial dew, followed by an additional dew period sufficient to bring the total incubation period to 24 hr. Leaves were sampled as described above. Experiments were conducted twice.

RESULTS

Influence of leaf wetness duration prior to dry periods. Plants given an initial dew period of 2, 4, 6, 8, 10, or 12 hr in the dew chamber, then a 2-hr dry period, followed by a variable dew period to complete the 24-hr incubation period, had an average of 283, 219, 152, 242, 261, or 282 lesions per plant after 2, 4, 6, 8, 10, or 12 hr, respectively, of initial dew period. Control plants held for 24 hr in the dew chamber had 383 lesions per plant. Since a dry period after 6 hr of initial dew had the fewest lesions, a 6-hr initial dew period was used in examining the effect of dry period duration on lesion production.

Influence of dry period duration on lesion production. Inoculated onion plants placed for 6 hr in a dew chamber, transferred to a growth chamber for dry interruption durations of 0.3–1.7 hr, and then returned to the dew chamber for the remainder of the 24-hr incubation period, had significantly fewer lesions than control plants that remained continuously in the dew chamber ($P = 0.05$) (Fig. 1). There was a tendency toward decreasing lesion numbers as the length of the dry period increased. Regression was significant (F test) at $P = 0.10$ but not at $P = 0.05$ ($R^2 = 56\%$).

Plants provided with extended dry periods of 4, 8, or 24 hr had fewer ($P = 0.05$) lesions than those provided with continuous wetness (Fig. 2). Regression was significant (F test) at $P = 0.10$ but not at $P = 0.05$ ($R^2 = 32\%$). There was a tendency toward decreasing lesion numbers as the dry period increased.

Influence of humidity during dry periods. Onion plants given a 6-hr period in the dew chamber, a 20-min dry period at 90, 60, or 30% RH, followed by 18 hr in the dew chamber had fewer lesions as humidities during the dew interruption were decreased (Fig. 3). Regression was significant (F test) at $P = 0.10$ but not at $P = 0.05$ ($R^2 = 25\%$).

Influence of dew period duration and timing of dry period on spore germination and infection. To determine directly the effects

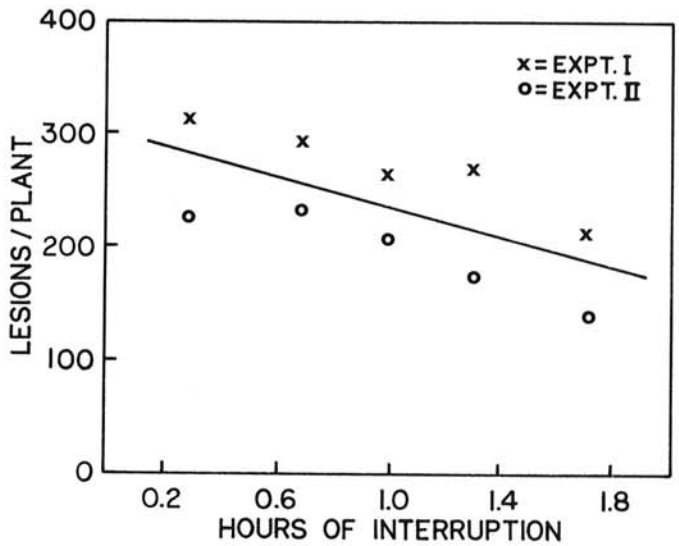

Fig. 1. Effect of dry period duration on number of lesions produced on onion by *Botrytis squamosa*. Plants were given a 6-hr postinoculation wetness period, a variable dry period, and a resumed wetness period to total a 24-hr treatment duration. Regression equation: $Y = 302 - 67.2\,X$. Control (continuous dew) values were 343 and 450 lesions per plant respectively.

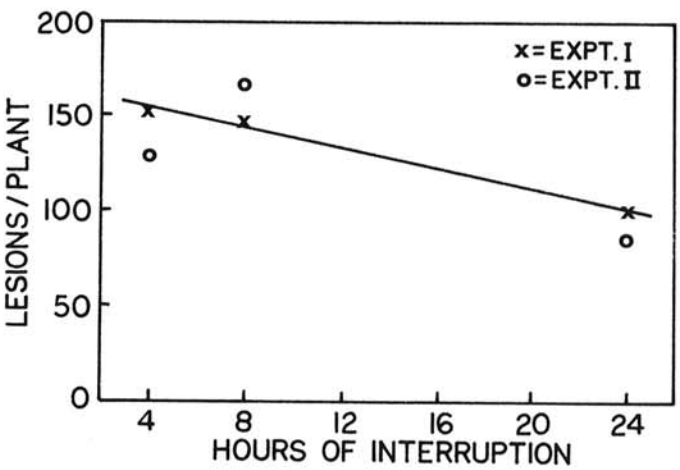

Fig. 2. Influence of 6 hr of postinoculation leaf wetness at 20 C, a variable dry period, and an additional 24-hr dew period on number of lesions produced on onion by *B. squamosa*. Regression equation: $Y = 161 - 2.5\,X$. Control (continuous dew) values were 413 and 380 lesions per plant respectively.

of dew period and of dew period interruption on the fate of germinating and penetrating conidia, we sampled, fixed, stained, and microscopically examined leaf samples from inoculated onion plants. Increasing duration of continuous dew periods resulted in increasing conidial germination, appressorium formation, and numbers of lesions through 12 hr of continuous dew and in increasing numbers of visible infection hyphae through 24 hr of continuous dew (Fig. 4). Regression of germination on dew period was significant at $P = 0.10$ ($R^2 = 41.3\%$), regression of appressorium formation on dew period was significant at $P = 0.05$ ($R^2 = 67.6\%$), regression of numbers of lesions on dew period was significant at $P = 0.05$ ($R^2 = 69.3\%$), and regression of visible infection hyphae on dew period was significant at $P = 0.01$ ($R = 76.7\%$).

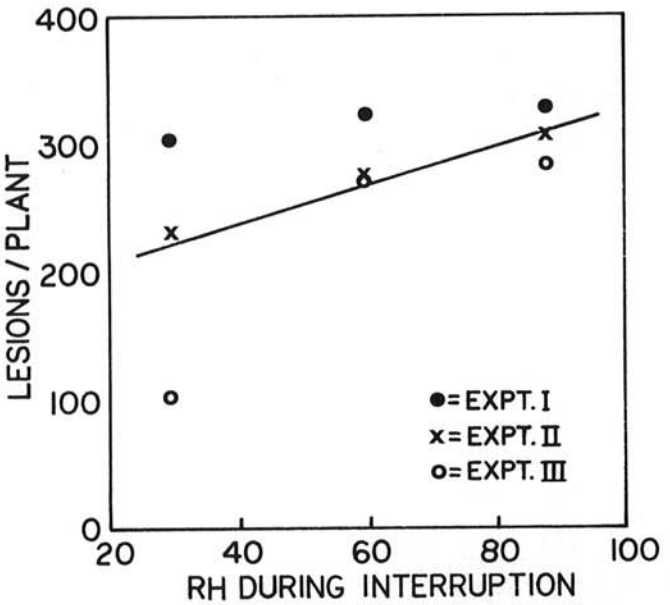

Fig. 3. Influence of a 6-hr postinoculation dew period, a 20 min dry period at 30, 60, or 90% RH, then an 18-hr dew period on numbers of lesions produced on onion by *B. squamosa*. Regression equation: $Y = 176 + 1.5\ X$. Control values (continuous dew) were 388, 426, and 376 lesions per plant respectively.

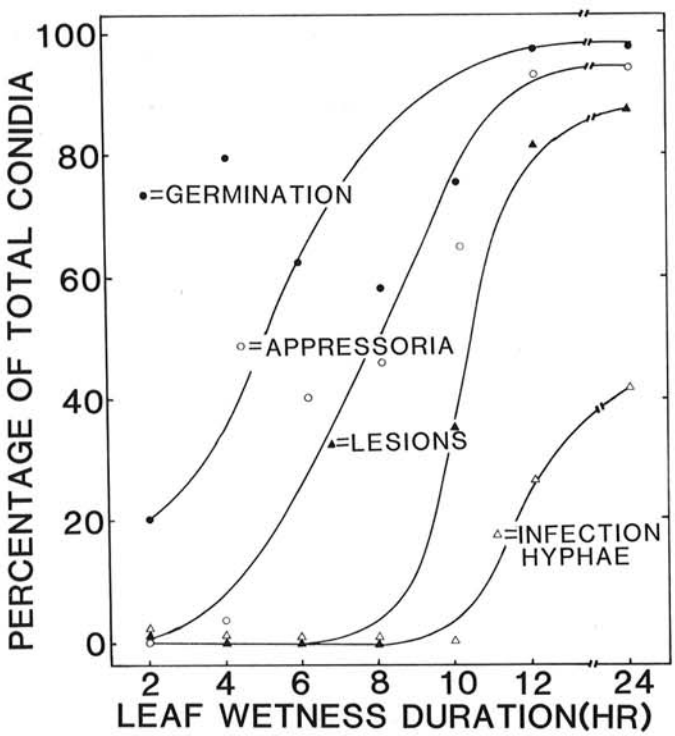

Fig. 4. Effect of variable dew periods on percent conidial germination, appressorium formation, lesions, and visible infection hyphae (all expressed as percentages of total conidia counted on leaf samples). Regression equations were: for germination, $Y = 45.0 + 2.6\ X$; for appressorium formation, $Y = 6.3 + 4.5\ X$; for lesions, $Y = 15.4 + 4.7\ X$; and for visible infection hyphae, $Y = -10.4 + 2.14\ X$.

Interruption of dew period for 2 hr did not significantly affect percent conidial germination, regardless of the timing of the interruption (Fig. 5). Interruptions after 6-hr initial dew periods resulted in the largest reductions in percent appressoria and lesions formed, and interruptions after 6 or 8 hr resulted in greatest reductions in percent visible infection hyphae (expressed as percent of total conidia counted on the leaf sample).

DISCUSSION

Plants given a 6-hr initial dew period followed by a 2-hr dry period and resumption of dew for the remainder of the 24-hr incubation period had fewer lesions than plants given a 2-, 4-, 8-, 10-, or 12-hr initial dew period. These data suggested that germinating spores were particularly vulnerable to drying after about 6 hr of dew, when germination had been initiated but before most germ tubes were protected by having penetrated the leaf, and we confirmed this hypothesis later with more detailed experiments (Fig. 5). The 6-hr initial dew period was then used to examine the effect of dew interruption duration on lesions produced. Even short dry interruptions following a 6-hr dew period reduced numbers of lesions on onion leaves, and numbers of lesions continued to decline with increasing lengths of dry periods (Figs. 1 and 2) in more or less linear fashion. This indicates that dry periods following the minimal length of dew period necessary for any visible lesions to occur (1,8) reduced infection efficiency at an increasing rate as dry periods lengthened, and underscores the epidemiological importance of uninterrupted leaf wetness periods. Our data generally support those of previous studies (4,6,10) with some quantitative refinements. Rather surprisingly, RH during a short 20-min dew interruption seemed to have some effect on lesion production (Fig. 3).

Our histogical observations on leaf samples revealed some interesting aspects of the effects of dew period and dry interruption

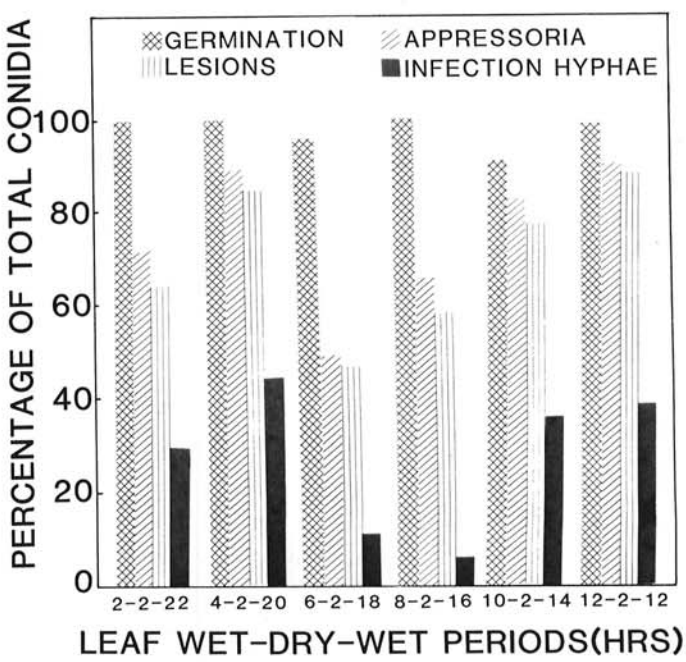

Fig. 5. Effect of timing of a 2-hr interruption of dew on percent conidial germination, appressorium formation, lesions, and visible infection hyphae (all expressed as percentage of total conidia counted on leaf samples).

period timing on the dynamics of spore germination, appressorial formation, lesion numbers, and visible infection hyphae. As expected, increasing dew period duration led to increasing percent conidia germinated, appressoria formed, lesions formed, and visible infection hyphae, and the time sequence of these events was clearly visible (Fig. 4). Conidial germination occurs first, followed by appressorium formation, then visible lesions, and finally visible infection hyphae within the lesions. Numbers of lesions were usually about 10% lower than percent conidia germinating, indicating that most germinating conidia establish lesions. The reasons for the low numbers of infection hyphae within lesions are obscure, but we (1) and others (3,7) have noted that the majority of the lesions of *B. squamosa* do not expand beyond a certain size (1–2 × 3-5 mm), and that the proportion of those in which hyphae continue to develop and lesions expand, leading to leaf blighting, is influenced by the length of continuous dew periods (1,3). The failure of *B. squamosa* to continue to grow in many lesions is suggestive of a hypersensitive-type reaction, and is supported by the generally recognized difficulty of isolating *B. squamosa* from lesions. Infection hyphae of *B. squamosa* grew about 3 times faster in senescent than in healthy onion leaf tissue (2), further suggesting that healthy onion tissue is resistant to ramification of infection hyphae.

The reduction in percent appressoria, lesions, and visible infection hyphae by dry periods occurring about 6–8 hr after onset of the initial dew period confirmed our hypothesis that, at these times, most conidia have germinated but have not yet penetrated and formed lesions (Fig. 4), and that the germ tubes are very likely vulnerable to drying if they have not yet penetrated. In our microscopic observations, we noted that some conidia formed appressoria and penetrated beneath the conidium with little or no germ tube visible, and these conidia may be less vulnerable to drying than those that form longer germ tubes. The timing and lengths of dry periods following onset of dew would thus seem to be important influences on numbers of lesions formed from a given inoculum load.

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