Ecology and Epidemiology

The Role of Temperature and Free Moisture in Onion Flower Blight

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ABSTRACT


The cardinal temperatures at which onion umbels were blighted (after inoculation when two-thirds of the florets were open) with Botrytis squamosa, B. cinerea, and B. allii (isolated from blighted onion florets) were near 9, 21, and 27 C for B. squamosa, near 12, 21, and 30 C for B. cinerea, and near 9, 24, and 30 C for B. allii. The cardinal temperatures for mycelial growth (potato-dextrose agar) of B. squamosa, B. cinerea, and B. allii were near 5, 22, and 30 C for each fungus. The cardinal temperatures for conidial germination (on purified water agar) were near 6, 15, and 30 C for B. squamosa; 3, 18, and 33 C for B. cinerea; and 6, 24, and 33 C for B. allii.

When the duration of free moisture on umbels after inoculation with the three pathogens was increased from 0 to 96 hr, the percentages of unopened florets, open florets, and immature seed capsules blighted at 21 C were increased significantly. Free moisture durations of 12-24, 6-12, and 6-12 hr were necessary for infection and 36-48, 12-24, and 24-36 hr were necessary for blighting of unopened florets, open florets, and immature seed capsules, respectively, by each pathogen at 21 C. A positive correlation between the amount of July rainfall and the natural incidence of onion flower blight was observed in Orange County, New York, from 1976 to 1981.

During 1975 and 1976, epidemics of flower blight of onion (Allium cepa L.) occurred in seed production fields in Orange County, New York (4,8). Onion flower blight in Orange County is caused by a pathogen complex consisting of Botrytis squamosa Walker, B. cinerea Pers. ex Fr., and B. allii Munn (B. allii may be a taxonomic synonym of B. aclada Fres.). Blighting by each species is characterized by necrosis of florets and immature seed capsules on onion umbels (4,7,8). Onion umbels covered by the spate of unopened umbels are less susceptible than open umbels to blighting by each of the three Botrytis species (7). Open umbels differ in susceptibility to each of the three Botrytis species depending on the proportion of florets that are unopen, open, or have developed into immature seed capsules. Unopened florets and immature seed capsules are more resistant to blighting by either B. squamosa, B. cinerea, or B. allii than are open florets (7). Resistance of certain cultivars of grape (Vitis labrusca L.) to B. cinerea can be overcome by increasing the duration of free moisture on plants (6). Therefore, it was postulated that at least part of the lower susceptibility of unopened onion florets and immature seed capsules relative to open florets might be due to longer durations of free moisture required for B. squamosa, B. cinerea, or B. allii to blight unopen florets or immature seed capsules.

Moisture is one of the primary factors associated with pathogenesis by B. squamosa and B. cinerea (6,9). The only two epidemics of onion flower blight that were observed in Orange County, New York, from 1975 to 1981 occurred when rainy conditions prevailed during the flowering period (4,8). Knowledge on the role of free moisture in onion flower blighting is needed to better understand the epidemiology of the disease.

The current study was conducted to determine the influence of temperature on conidial germination, mycelial growth, and blighting of onion umbels for B. squamosa, B. cinerea, and B. allii and to evaluate the relationship of free moisture to blighting of onion florets at different stages of development by each of the three pathogens.

MATERIALS AND METHODS

Inoculum production. Most of the isolates of B. squamosa, B. cinerea, and B. allii utilized in this study were from blighted onion florets collected in Orange County. One exception was B. squamosa isolate 24-1, which was obtained from a single ascospore produced in culture.

Inoculum used for the studies conducted to investigate the relationship between temperature and mycelial growth was produced by first transferring a single conidium of each isolate to the center of a plastic petri dish (9-cm diameter) containing 15 ml of potato-dextrose agar (PDA; Difco Laboratories, Detroit, MI). The dishes then were sealed with Parafilm and incubated at 20 C in the dark.

One-day-old conidia of B. cinerea and B. allii were used to investigate the role of temperature in conidial germination and flower blight and the role of free moisture in flower blight. Single conidia of B. cinerea and B. allii from stock cultures first were transferred to the center of plastic petri dishes (9-cm diameter)
containing 15 ml of PDA. The dishes then were sealed with Parafilm and incubated for 7-10 days and placed in an incubator at 21 °C under fluorescent lights (Sylvania F20T12/CW; Sylvania Lighting Center, Danvers, MA) with a 14 hr photoperiod. One day before inoculation was required, the conidia present were removed carefully from the conidiophores by using a sterile vacuum apparatus connected to a faucet aspirator. The dishes then were resealed and returned to the incubator for 24 hr, after which newly formed conidia were collected into 100 ml of sterile distilled water with one drop of Tween 20. Suspensions of 30,000 conidia per milliliter were used for all experiments.

The isolates of *B. squamosa* utilized sporulated poorly on PDA and, therefore, conidia were produced in cultures growing on onion straw agar (7). Virolence of *B. squamosa*, *B. cinerea*, and *B. allii* was maintained by mononoidial transfer and passage through onion florets on alternate transfers.

**Temperature and mycelial growth.** The growth of mycelium of three isolates of *B. squamosa* (BS24-1, BS80-2, and BS80-6), *B. cinerea* (BC80-3, BC80-6, and BC81-1), and *B. allii* (BA80-1, BA80-3, and BA81-2) was studied at temperatures of 30°C (5-C intervals). Disks 5 mm in diameter were cut with a sterile cork borer from the colony perimeter of 7-day-old mononoidial cultures. The disks were placed in the center of plastic petri dishes (9-cm diameter) containing 15 ml of PDA and sealed with Parafilm. The dishes were incubated in the dark at 30°C. Colony radius was measured after 3 days (five replicates). After the optimum growth temperatures for the three *Botrytis* species were determined at 5-C intervals, the experiment was repeated at 2-C intervals 4 C above and 2 C below the previously determined (5-C intervals) optimum temperature.

**Temperature and conidial germination.** Conidial germination of three isolates of *B. squamosa*, *B. cinerea*, and *B. allii* was studied at a range of temperatures from 3 to 36°C at 3-C intervals, using the same isolates as in the mycelial growth study. Aliquots (5 ml) of conidial suspensions of each isolate were poured over the surface of 15 ml of purified water 5% agar (Difco Laboratories, Detroit, MI) in plastic petri dishes (9-cm diameter). After 5 min, the water was poured off and the dishes were incubated at the appropriate temperatures. Conidial germination was assessed by counting the number of germinated conidia among 500 conidia observed. These counts were performed at 12 and 24 hr by randomly adjusting the position of the petri dishes on the stage of a microscope. A conidium was considered germinated if the germ tube equaled or exceeded the length of the conidium.

**Inoculation.** Inoculation experiments were conducted to demonstrate the relationships between temperature and onion flower blight and between free moisture and onion flower blight. All inoculation experiments used onion umbels (cultivar Sentinel; Joseph Harris Company, Rochester, NY) that were excised from greenhouse-grown plants with approximately 30 cm of scape attached. The scapes were recut under tap water and placed in 250-ml Erlenmeyer flasks of tap water (7).

Conidial suspensions were sprayed onto umbels until the surfaces were covered with a fine mist by using a Prevail aerosol atomizer (Precision Valve Corporation, Yonkers, NY). Inoculation, umbels were first incubated in a dew chamber (9) at different temperatures and for different durations depending on the experiment, and then were moved to a walk-in growth chamber at 24°C day, 18°C night with a 16-1 hr photoperiod (20 kilolux).

**Temperature and onion flower blight.** The relationship between temperature and the amount of blighting of onion florets by the three *Botrytis* species was studied during continuous free moisture on umbel surfaces. Onion umbels (five replicates) with approximately two-thirds of the florets open were inoculated with conidial suspensions of either *B. squamosa* (isolate BS80-6), *B. cinerea* (isolate BC80-3), or *B. allii* (isolate BA80-1). The umbels then were placed in the dew chamber for 72 hr at different temperatures ranging from 9 to 30°C (3-C intervals). Disease was assessed 10 days after inoculation by counting the number of blighted and healthy florets on the umbels and then calculating the percentage of blighted florets. An uninoculated control was included with the inoculated umbels incubated at each temperature.

**Duration of free moisture and onion flower blight.** The relationship between the duration of free moisture and the amount of blighting of onion florets by the three *Botrytis* species was determined on unopened florets, open florets before pollen shed, and immature seed capsules. Pollination of florets and subsequent production of seed capsules was conducted on onion plants in the greenhouse (7). Ten florets at each development stage were marked on each umbel (five replicates). Umbels were inoculated with either *B. squamosa* (isolate BS80-6), *B. cinerea* (isolate BC80-3), or *B. allii* (isolate BA80-1). Inoculations with each pathogen were conducted separately due to limited space in the dew chamber. An uninoculated control and an inoculated control, placed in the walk-in growth chamber instead of in the dew chamber, were included with each set of inoculated umbels. Inoculated umbels were placed in the dew chamber and five umbels removed after 6, 12, 24, 36, 49, 3, 72, and 96 hr. Disease was assessed by counting the number of collared florets at each development stage that were blue within 10 days after inoculation (Fig. 1C).

To determine if symptomless florets were infected, fungal isolations were made from artificially inoculated florets and seed capsules. Twenty-five collared florets of each development stage, inoculated with each pathogen and exposed to each duration of free moisture, were surface-disinfested for 3 min in 0.5% sodium hypochlorite solution (one drop of Tween 20 per 100 ml). The florets were plated on 15 ml of acidified PDA (APDA) in plastic petri dishes (9-cm diameter). The dishes were sealed with Parafilm and incubated at 21°C for 7-10 days under fluorescent lights (Sylvania F20T12/CW; Sylvania Lighting Center, Danvers, MA). The dishes were examined to determine if any of the three pathogens had grown from the symptomless florets.

**Environmental data and disease monitoring.** Temperature and rainfall were recorded on a continuous basis for the months of June through August during 1976-1981 at a weather monitoring station in Orange County near Florida, New York. This station was located in an onion bulb production field within 2.5 km of all the onion seed fields monitored for onion flower blight from 1976 to 1981.

Incidence of onion flower blight in Orange County seed production fields was assessed by selecting 500 umbels at random in each field and calculating the percentage of umbels with symptoms of flower blight. Blight was assessed between 12 and 20 July in seven fields in 1976, 11 fields in 1977, and 10 fields in 1978-1981. The most common cultivars in the fields were homegrown, open-pollinated Early Yellow Globe and Early Yellow Medium. Due to crop rotation, it was not always possible to monitor the same fields each year.

**Statistical analysis.** Data from the free moisture studies were analyzed by using linear regression (Minitab, The Pennsylvania State University, University Park). Data points recorded were the mean of five replicates.

**RESULTS**

**Temperature and mycelial growth.** The cardinal temperatures for mycelial growth were near 5, 22, and 30°C for all three *Botrytis* species and the temperature response curves were of similar shape (Fig. 1A and B). At the range of temperatures tested, radial colony growth was greater for *B. cinerea* than for *B. allii* and *B. squamosa*, which were similar. For each *Botrytis* species the cardinal temperatures were the same for the three isolates tested.

**Temperature and conidial germination.** The cardinal temperatures for conidial germination were near 6, 15, and 30°C for *B. squamosa*, near 3, 18, and 33°C for *B. cinerea*, and near 6, 24, and 33°C for *B. allii* (Fig. 1C). The response curves for the three species were of different shapes. Conidia of *B. cinerea* and *B. allii* germinated over a wider range of temperatures than did conidia of
B. squamosa. The cardinal temperatures were the same for the three isolates of each Botrytis species and the growth curves were similar in shape.

**Temperature and onion flower blight.** The cardinal temperatures for blighting of open florets were near 9, 21, and 27 C for B. squamosa, near 12, 21, and 30 C for B. cinerea, and near 9, 24, and 30 C for B. allii (Fig. 1D). The temperature response curves for the three Botrytis species were of different shapes, and the curves of B. cinerea and B. allii were skewed more toward warmer temperatures than the curve of B. squamosa. B. cinerea and B. allii blighted florets over a greater range of temperatures than B. squamosa.

**Duration of free moisture and onion flower blight.** Regression models indicated that duration of free moisture contributed significantly (P = 0.001) to the variation in the percentage of florets and immature seed capsules blighted by B. squamosa, B. cinerea, or B. allii (Fig. 2A to C). The best model for florets at each development stage consisted of two lines with significantly different (P = 0.01) slopes and intercepts. One line represented the percentages of florets blighted by B. squamosa, and the second line represented the percentages of florets blighted by B. cinerea and B. allii. The regression models for open florets (Fig. 2B), unopen florets (Fig. 2A), and immature seed capsules (Fig. 2C) indicated that there were no significant differences (P = 0.05) between separate lines representing the percentages of florets or immature seed capsules blighted by either B. cinerea or B. allii.

B. squamosa blighted greater percentages of open or unopen florets and immature seed capsules than did B. cinerea or B. allii at all durations of free moisture to which umbels were exposed (Fig. 2A to C). However, after open florets were exposed to 96 hr of continuous free moisture, B. squamosa had blighted 100% of the florets inoculated, and the percentages of florets blighted by B. cinerea and B. allii began to approach this level of blight. Blighting of florets was first observed after open florets had been exposed to 24 hr and unopen florets exposed to 48 hr of continuous free moisture. Blighting of immature seed capsules was observed after umbels were exposed to 36 hr of continuous free moisture. B. cinerea and B. allii required a longer period of free moisture than B. squamosa to cause a given level of blight.

B. squamosa, B. cinerea, and B. allii were isolated from open florets and immature seed capsules that has been exposed to 12 hr or more of continuous free moisture. Two Botrytis species were each isolated from unopen florets exposed to 24 hr or more of continuous free moisture. The three Botrytis species each were isolated from unopen florets exposed to 24 hr or more of continuous free moisture. Only saprophytic fungi were isolated (occasionally) from florets or seed capsules exposed to less than 6 hr of free moisture.

**Environmental data and disease monitoring.** The average temperature during July for each year from 1976 to 1981 was 19.4, 22.2, 21.3, 21.5, 21.5, and 22.7 C, respectively (Table 1). Average daily temperatures in July ranged 16.6-25.6, 16.1-27.8, 15.2-27.8, 16.4-26.5, 18.1-26.4, and 15.7-28.1 for each year from 1976 to

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Fig. 1. The relationship of temperature to colony radius, germination of conidia, and percent blighted florets for Botrytis squamosa, B. cinerea, and B. allii. A, Colony radius measured after 3 days of growth on potato-dextrose agar (5-C intervals). B, Colony radius measured after 3 days of growth on potato-dextrose agar. C, Germination of conidia after 24 hr on purified water agar. Data for colony radius and germination of conidia are averages of three isolates each of B. squamosa, B. cinerea, and B. allii. D, The percentages of florets blighted by one isolate of each pathogen 10 days after inoculation.
1981, respectively. The total July rainfall and incidence of onion flower blight at 1 and 10% levels in July of each year from 1976 to 1981 is shown in Table 1. Ten percent or more of the umbels in 100% of the seed fields monitored in July 1976 exhibited onion flower blight symptoms. No fields exhibited this level of blight from 1977 to 1981 (Table 1). The only onion flower blight epidemic that occurred from 1976 to 1981 was observed during July 1976.

**DISCUSSION**

Results of the current study indicated that the cardinal temperatures for mycelial growth of *B. cinerea*, *B. squamosa*, and *B. allii* isolated from blighted onion florets were near 5, 22, and 30 C. In most previous studies, the optimum temperature for growth of *B. cinerea* was 20–25 C (2). *B. cinerea* isolated from cabbage had an optimum temperature for growth on PDA of 20–25 C, while the optimum temperature for infection and decay of cabbage was 20 C (10). Upper and lower limits for growth on PDA and on cabbage were 0 and 30 C, respectively. Optimum temperature for growth of *B. squamosa* on PDA was 24 C (9). Optimum temperature for pathogenesis of *B. squamosa* on onion leaves (lesion formation) is probably lower than the optimum temperature for mycelial growth (9). The temperature range at which lesion formation occurred was less (9–23 C) than that for mycelial growth (9–31 C). In the current study, in concert with the report of Shoemaker and Lorbeer (9), *B. squamosa* was demonstrated to possess a lower optimum temperature for blighting of onion florets (20 C) than for mycelial growth on PDA (22 C). Similarly, and in agreement with the report of Yoder and Whalen (10), *B. cinerea* had a lower optimum temperature for blighting onion florets (21 C) than for mycelial growth on PDA (22 C). Mycelium of *B. allii* grew at 8–40 C, with most rapid growth near 40 C on onion agar (5). In the current study, the optimum temperature for *B. allii* to blight onion florets was higher (24 C) than the optimum temperature for mycelial growth on PDA (22 C).

For *B. squamosa*, *B. cinerea*, or *B. allii*, the differences between optimum temperatures for blighting onion florets and for mycelial growth in the current study may be due at least in part to differences in temperature requirements for germination of conidia. For *B. squamosa* and *B. cinerea*, the optimum temperatures for germination of conidia (15 and 18 C, respectively) were lower than the optimum temperature for mycelial growth (22 C). For *B. allii*, the optimum temperature for germination of conidia (24 C) was higher than the optimum temperature for mycelial growth (22 C). Conidia of *B. allii* and *B. cinerea* germinated over a wider range of temperatures than *B. squamosa*. Conidia of *B. squamosa* isolate 64a (a mutant isolate) germinated from 12 to 27 C, with maximum germination near 15 C (9). We also found 15 C to be the temperature for maximum germination of conidia of *B. squamosa*. However, the range of temperatures at which germination occurred was from near 6 to 30 C. Botton (3) reported that conidia of *B. cinerea* germinated from near 5 to 30 C, with a maximum near 20 C. In the current study, conidia of *B. cinerea* germinated from near 3 to 33 C, with a maximum near 18 C. Conidia of *B. allii* germinated from near 6 to 33 C, with a maximum near 24 C.

**TABLE 1.** Rainfall and average temperature in July and incidence of onion flower blight in Orange County, New York, onion seed fields from 1976 to 1981

<table>
<thead>
<tr>
<th>Year</th>
<th>Onion flower blight incidence*</th>
<th>July</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1%</td>
<td>10%</td>
</tr>
<tr>
<td>1976</td>
<td>100</td>
<td>100</td>
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<tr>
<td>1977</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>1978</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>1979</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>1980</td>
<td>40</td>
<td>0</td>
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*Data are the percentages of the fields sampled each year in which a minimum of 1 or 10% of the umbels were visibly blighted. The blight assessments were performed between 12 and 20 July each year.

**Fig. 2.** Relationship between duration of free moisture and percentages of onion florets at three different development stages blighted by either *B. squamosa*, *B. cinerea*, or *B. allii* (one isolate of each pathogen). Florets at the desired development stage were marked with Parafilm collars and umbels were inoculated with conidial suspensions (30,000 conidia per milliliter). Data points are the mean percentages of florets blighted at each development stage by each pathogen (five umbels, 10 florets per umbel). Dotted lines are regression lines. A, Unopened florets ($R^2 = 74\%$, slopes = 0.4 and 0.2). B, Open florets ($R^2 = 94\%$, slopes = 1.2 and 0.9). C, Immature seed capsules ($R^2 = 81\%$, slopes = 0.9 and 0.3).
The average daily temperatures recorded in Orange County during the onion flowering period (July) were within the range of temperatures at which B. squamosa, B. cinerea, and B. allii blighted onion florets. In the current study, the average temperature during July 1976 was 2 °C cooler than for July in 1977–1980. B. squamosa was isolated from blighted onion florets more frequently during 1976 than during 1977–1981 (8). Thus, it is interesting to note that B. squamosa, which was more virulent than B. cinerea or B. allii at cooler temperatures, was isolated most frequently from blighted florets during the coolest of the six July flowering periods (July 1976). This suggests that under cooler average temperatures, B. squamosa may be more important in causing onion flower blight than the other two species of Botrytis.

Moisture is a primary factor regulating plant diseases caused by B. squamosa and B. cinerea. A minimum of 12–24 hr of free moisture is necessary for infection of the fruit of cultivar Tokay grape by B. cinerea (12 C night, 21 C day, 16 C average) (6). A minimum 6 hr of free moisture is necessary for B. squamosa (isolate 64a) to produce lesions on onion leaves (20 C) (9). Results of the current study indicated that between 6 and 24 hr of free moisture were necessary for infection of onion florets and immature seed capsules by either B. squamosa, B. cinerea, or B. allii and that from 12 to 48 hr were necessary for blighting of the florets or immature seed capsules (21 C).

Open florets, unopened florets, and immature seed capsules differed in the duration of free moisture necessary for infection and blighting by each pathogen in the dew chamber. The required periods of free moisture in the dew chamber were the same for each of the pathogens on either unopened florets, open florets, or immature seed capsules. Open florets were the most susceptible of the development stages and required from 6 to 12 hr of continuous free moisture for infection and from 12 to 24 hr for blighting. Immature seed capsules required the same period of continuous free moisture for infection and 24–36 hr for blighting. Unopened florets were the least susceptible development stage and required from 12 to 24 hr of continuous free moisture for infection and 36 to 48 hr for blighting. These observations are in concert with the report of Nelson (6) that a longer duration of free moisture was necessary for infection of the fruit of a more resistant grape cultivar compared to a more susceptible cultivar by B. cinerea.

An increased percentage of unopened florets, open florets, and immature seed capsules were blighted by B. squamosa, B. cinerea, and B. allii as the duration of free moisture to which umbels were exposed in the dew chamber was increased. These results agree with the review by Blakeman (2) that increased periods of free moisture have been related to increased incidence of leaf infections by B. cinerea and other Botrytis species. The results also agree with the report (9) that increasing durations of free moisture resulted in an increase in the number of lesions on onion leaves formed by B. squamosa (isolate 64a). In the current study, when inoculated umbels were exposed to increasing durations of continuous free moisture in the dew chamber, B. squamosa consistently blighted a greater percentage of florets and immature seed capsules than B. cinerea or B. allii. However, after 96 hr of free moisture, B. squamosa had blighted 100% of the marked open florets and B. cinerea and B. allii had caused levels of blight approaching 100%. These observations suggest that provided with a long enough duration of free moisture, B. cinerea, B. allii, and B. squamosa would produce an equivalent amount of blighting of open florets.

Free moisture is necessary for lesions of Sclerotinia sclerotiorum on bean flowers to expand (1). In the current study the increase in the percentages of blighted open florets and immature seed capsules that occurred due to exposure to increased periods of free moisture (in the dew chamber) may have been due to either a greater number of infections taking place or a requirement of the pathogens for free moisture in order to colonize uninfected tissue. Longer periods of free moisture may have allowed more conidia to germinate and/or provided more time for hyphae to locate suitable sites for infection.

The dependence of B. squamosa, B. cinerea, and B. allii on long periods of continuous free moisture (24 hr or more) to induce a substantial amount of blighting of florets and immature seed capsules explains the positive correlation between rainfall and onion flower blight incidence observed in Orange County during the relatively rainy July 1976 and the relatively dry months of July during 1977–1981.

LITERATURE CITED