Pathogenicity of *Botrytis* Species on Onion Umbels and Scapes Under Controlled Conditions

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ABSTRACT

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Botrytis squamosa blighted a higher percentage of onion florets than either *B. cinerea* or *B. allii* when umbels with all the florets unopen or umbels with approximately two-thirds of the florets open were inoculated in a dew chamber. *B. byssoidea* was nonpathogenic under these conditions. *B. squamosa* blighted more florets than *B. cinerea* or *B. allii* at all inoculum concentrations tested. There were no differences in percentages of blighted florets for the open-pollinated onion cultivars Early Yellow Globe, Early Yellow Medium, and Italian Red and the hybrid cultivars Ontario M and Sentinel. Onion umbels covered by the spathe were susceptible only to *B. squamosa*. The susceptibility of open umbels to either *B. squamosa*, *B. cinerea*, or *B. allii* increased as the proportion of open to unopen florets at the time of inoculation was increased. Open florets were more susceptible than were unopen florets or immature seed capsules. Blighting was not increased by inoculating two of the pathogens together.

Additional key words: *Allium cepa*, epidemiology, etiology.

Blighting of the umbels of onion (*Allium cepa* L.) by *Botrytis* species has been reported in Idaho by Blodgett (1), in Connecticut by Clinton (3), in Israel by Netzer and Dishon (10), and in New York by Ellerbrock and Lorbeer (4). During the rainy summer of 1975 in Orange County, New York (475 mm total rainfall in June, July, and August), Ellerbrock and Lorbeer (4) demonstrated that *B. cinerea* Pers. ex Fr., *B. squamosa* Walker, and *B. allii* Munn (*B. allii* may be a taxonomic synonym of *B. aclada* Fres.) inoculated onto onion umbels in the field blighted onion florets and seed capsules and thereby reduced seed yield by 98, 93, and 48%, respectively, compared to an uninoculated control. *B. cinerea*, *B. squamosa*, *B. allii*, and *B. byssoidea* Walker were isolated from naturally blighted onion florets in Orange County, New York, during 1975 (4) and during 1976–1981 (12).

Grape (*Vitis labrusca* L.) and strawberry (*Fragaria chiloensis* Duchesne var. *ananassa* Bailey) flowers differ in susceptibility to *B. cinerea* according to their stage of development (8,9). The onion umbel has a complex sequence of development. Initially the onion umbel is covered by the spathe. As the umbel expands, the spathe is broken and the still unopen florets are exposed. The florets then open over a period of approximately 2 wk. It was postulated that open umbels might differ from umbels covered by the spathe in susceptibility to one or more of the *Botrytis* species. It also was postulated that open umbels might differ in susceptibility to one or more of the *Botrytis* species due to differences in susceptibility of unopen florets, open florets, and immature seed capsules.

The study reported here was conducted to determine pathogenicity and virulence of *Botrytis* species, singly or in dual inoculations, and at different inoculum concentrations; cultivar susceptibility; and flower and immature seed capsule susceptibility at different stages of development.

MATERIALS AND METHODS

Inoculum production. One-day-old conidia of *B. cinerea* and *B. allii* were produced by first transferring single conidia to plastic petri dishes (9-cm diameter) containing 15 ml of potato-dextrose agar (PDA; Difco Laboratories, Detroit, MI). The dishes were sealed with Parafilm and placed in an incubator at 21 C under fluorescent lights (Sylvania F20T12/CW; Sylvania Lighting Center, Danvers, MA) with a 14-hr photoperiod for 7–14 days. One day before inoculum was required, the conidia present were carefully removed from conidiophores by using a sterile vacuum apparatus connected to a faucet aspirator, and then the dishes were resealed and returned to the incubator for 24 hr. Newly formed conidia were collected with the sterile vacuum apparatus into 100 ml of sterile distilled water which contained one drop of Tween 20 to assure dispersal of the dry conidia in the water. One-day-old conidia of *B. byssoidea* were produced and collected by a similar method except that onion straw agar (15 ml of 2.0% water agar poured over enough dried onion straw to cover the bottom of a 9-cm-diameter glass petri dish and then autoclaved) was utilized.

Conidia of *B. squamosa* for use in inoculation experiments were produced by first transferring single conidia to 9-cm-diameter plastic petri dishes containing 15 ml of PDA. The dishes were sealed and incubated at 21 C for 10–14 days until a mycelial mat covered the surface of the medium. The dishes were either utilized or stored at 1 C for use at a later time. To produce conidia, an entire 9-cm-diameter agar disk covered with mycelium of *B. squamosa* was inverted onto the surface of a dish of onion straw agar. Pressure then was applied to the back of the agar disk with a scalpel so that the mycelium on the front of the disk contacted as large a surface area of the onion straw as possible. The disk then was removed and used to seed a second dish of straw agar with *B. squamosa*. The two straw agar dishes then were sealed with Parafilm and incubated at 14 C under continuous UV blue light (Sylvania F20T8/BLB; Sylvania Lighting Co., Danvers, MA) for 8–10 days until conidia had formed. Conidia used as inoculum were collected from the straw agar with the sterile vacuum apparatus.

Virulence of *B. squamosa*, *B. cinerea*, and *B. allii* was maintained by monoconidial transfer and passage through onion florets on alternate transfers. In all experiments, except those designed to investigate the virulence of different isolates of each *Botrytis* species, the isolates utilized were obtained from blighted onion florets collected in Orange County. Unless otherwise specified, all suspensions of conidia used in inoculation experiments were adjusted to 30,000 conidia per millilter.

Inoculation procedure. Onion umbels with approximately 30 cm of scape attached were excised from greenhouse-grown plants. The

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scape ends were recut in a dishpan full of tap water, and while still submerged in the water the scapes were transferred into submerged 250-ml Erlenmeyer flasks. Umbels treated in this manner developed normally for at least 14 days, which allowed sufficient time for inoculation and disease assessment. Excised umbels did not differ from intact umbels in susceptibility to each of the four *Botrytis* species.

Onion umbels at different stages of development were designated as stages 1–5. These were: stage 1—umbel covered by the spathe, stage 2—umbel open but all florets unopen, stage 3—umbel open with approximately one-third of the florets open, stage 4—umbel open with approximately two-thirds of the florets open, stage 5—umbel open and all florets open.

Conidial suspensions were sprayed, by using a Preval aerosol atomizer (Precision Valve Corporation, Yonkers, NY), onto umbels until the surfaces of the florets were covered with a fine mist. After inoculation, the umbels were incubated for 72 hr at 21 C in a dew chamber (13) and then were moved to a walk-in growth chamber maintained at 24 C day and 18 C night with a 16-hr photoperiod (20 kilolux). Ten days after inoculation, blighted and nonblighted florets on each umbel were counted. In each experiment, inoculations with each *Botrytis* species were conducted separately due to limited space in the dew chamber and the potential for contamination. An uninoculated control was included with each set of umbels that were inoculated.

Variation in virulence of isolates. Umbels (cultivar Early Yellow Globe) at stages 2 and 4 (five replicates) were inoculated with five isolates each of *B. squamosa*, *B. cinerea*, *B. allii*, and *B. byssoidea* and incubated in the dew chamber. Ten blighted florets from umbels inoculated with each pathogenic isolate were surface-disinfested for 3 min in 0.5% sodium hypochlorite solution (one drop of Tween 20 per 100 ml) and plated on acidified PDA in plastic petri dishes to reisolate the pathogens.

The isolates of *B. squamosa* utilized were 64a (a mutant isolate that sporulates on PDA), BSS-2 (a Michigan isolate that sporulates on PDA), 24-1 (a laboratory culture originally obtained from a single ascospore), and BS80-2 and BS80-6 (wild types isolated from blighted onion florets collected in Orange County during 1980). The isolates of *B. cinerea* utilized were 61-34 (a laboratory culture originally obtained from an onion collected in New York) and BC76-4, BC77-2, BC80-3, and BC80-2 (wild types isolated from blighted onion florets collected in Orange County from 1976 to 1980). The isolates of *B. allii* utilized were BA-74 (a laboratory culture of unknown origin) and BA76-14, BA77-5, BA80-1, and BA80-3 (wild types isolated from blighted onion florets collected in Orange County from 1976 to 1980). The isolates of *B. byssoidea* utilized were BB79-1, BB79-3, BB80-1, BB80-2, and BB80-5 (wild types isolated from blighted onion florets collected in Orange County from 1979 to 1980).

Effect of inoculum concentration. Umbels (cultivar Early Yellow Globe) at stages 2 and 4 (five replicates) were inoculated with conidial suspensions of *B. squamosa*, *B. cinerea*, and *B. allii* each at concentrations of 0, 3,750, 7,500, 15,000, or 30,000 conidia per milliliter.

Susceptibility of onion cultivars. Homegrown, open-pollinated Early Yellow Globe, Early Yellow Medium, and Italian Red (three onion cultivars commonly grown for seed production in New York) and hybrid cultivar Ontario M (Asgrow Seed Co., Kalamazoo, MI) were tested for susceptibility to *B. squamosa*, *B. cinerea*, and *B. allii*. Stage 4 umbels (five replicates) were inoculated with each pathogen. Only stage 4 umbels were tested because limited numbers of plants were available. In a similar experiment, the susceptibility of cultivars Sentinel (Joseph Harris Company, Inc., Rochester, NY) and Early Yellow Globe was compared.

Susceptibility of umbels at the five development stages. Umbels at stages 1–5 were tested for susceptibility to *B. squamosa*, *B. cinerea*, *B. allii*, and *B. byssoidea*. Five umbels at each development stage were inoculated with each *Botrytis* species and placed in the dew chamber with five uninoculated umbels for 10 days. Owing to limited space in the dew chamber and to the danger of cross contamination, inoculations with each *Botrytis* species were conducted separately.

Susceptibility of unopen florets, open florets, and immature seed capsules. Four different floret development stages (unopen florets, open florets before pollen shed, open florets after pollen shed, and open florets as petals began to senesce) were identified on umbels with the majority of florets open (umbels between stages 4 and 5). These stages were likely to differ in susceptibility to one or more of the *Botrytis* species due to physical differences or differences in phyllosphere nutrient levels (e.g., presence of pollen or nectar). Parafilm collars (1-cm square) marked to identify the time of inoculation were placed around the pedicels of the florets (Fig. 1A).

To investigate the susceptibility of immature seed capsules, onion umbels intact on plants were placed in a screened chamber for 2–3 wk during which florets were pollinated by house flies (*Musca domestica* L.). When umbels composed entirely of immature seed capsules were obtained, five capsules of approximately equal age on each of five umbels were marked with Parafilm collars. Umbels with marked florets or immature seed capsules were inoculated with either *B. squamosa*, *B. cinerea*, or *B. allii*, incubated in the dew chamber, and assessed for disease 10 days after inoculation. Each inoculation was conducted twice.

Dual inoculations. Three experiments were conducted in which two of the pathogenic *Botrytis* species were inoculated onto umbels at stage 5. Umbels at this stage were utilized so that blighting would reflect pathogenesis by the *Botrytis* species on a large number of open florets. The presence of some senescent florets on stage 5 umbels was postulated to have less effect on pathogenesis than would the presence of unopen florets on stage 4 umbels. Each experiment consisted of two treatments in which umbels were inoculated with each pathogen alone (30,000 conidia per milliliter), two treatments in which inoculations were with one pathogen (30,000 conidia per milliliter) 48 hr before the other pathogen (30,000 conidia per milliliter), one treatment in which inoculations were with the two pathogens simultaneously (30,000 conidia of each pathogen per milliliter), and an uninoculated control. After inoculation, all umbels were incubated for 96 hr in the dew chamber and disease was assessed 10 days after inoculation. Thirty blighted florets from each treatment in each experiment were surface-disinfested and plated on acidified PDA in plastic petri dishes to reisolate the pathogens.

Histology. Microscopic observations of onion florets inoculated with either *B. squamosa*, *B. cinerea*, or *B. allii* were made to confirm the presence of the pathogens in or on inoculated tissue. Petals, stamens, and styles were excised, bisected transversely, and placed on glass microscope slides in drops of 2% glutaraldehyde prepared with Sorenson's phosphate buffer (pH 7.0). The tissue was fixed for 30 min, the glutaraldehyde was drawn off, and the tissue was rinsed three times with the buffer. Drops of 1% acid fuchsin or 1% cotton blue (both in lactophenol) were then placed over the tissue. The tissue was stained for 30 min, destained in lactophenol for 2 min, and mounted in 50% glycerol. The tissue was examined by using bright-field and phase-contrast optics.

Statistical analyses. A one-way analysis of variance (Minitab; The Pennsylvania State University, University Park) was utilized for all inoculation experiments except those designed for investigating inoculum concentration. Means were compared by using Fisher's Protected LSD Test ($P = 0.05$) or statistical contrasts (Statistical Analysis Systems; SAS Institute, Cary, NC). Linear regression (Minitab) was used to analyze data from the experiments designed to investigate the effects of inoculum concentration on blighting caused by each of the three *Botrytis* species.

RESULTS

Variation in virulence of isolates. Of those that were tested, all isolates of *B. byssoidea* were nonpathogenic, all isolates of *B. squamosa* were pathogenic, and there were no significant differences among the isolates in the percentages of florets blighted on umbels at either stage 2 or 4 (Fig. 2A). Isolates of both *B. cinerea* and *B. allii* blighted significantly different percentages of florets on umbels at these two stages (Fig. 2B and C). *B. cinerea* isolate 61–34 and *B. allii* isolate BA-74 were both nonpathogenic. These isolates

had been in culture for several years. The remaining isolates of both *B. cinerea* and *B. allii* were all pathogenic but differed in virulence. There was a trend for more recent isolates of both fungi to be more virulent than older isolates. However, even among isolates obtained recently, there were some significant differences in the percentages of florets blighted.

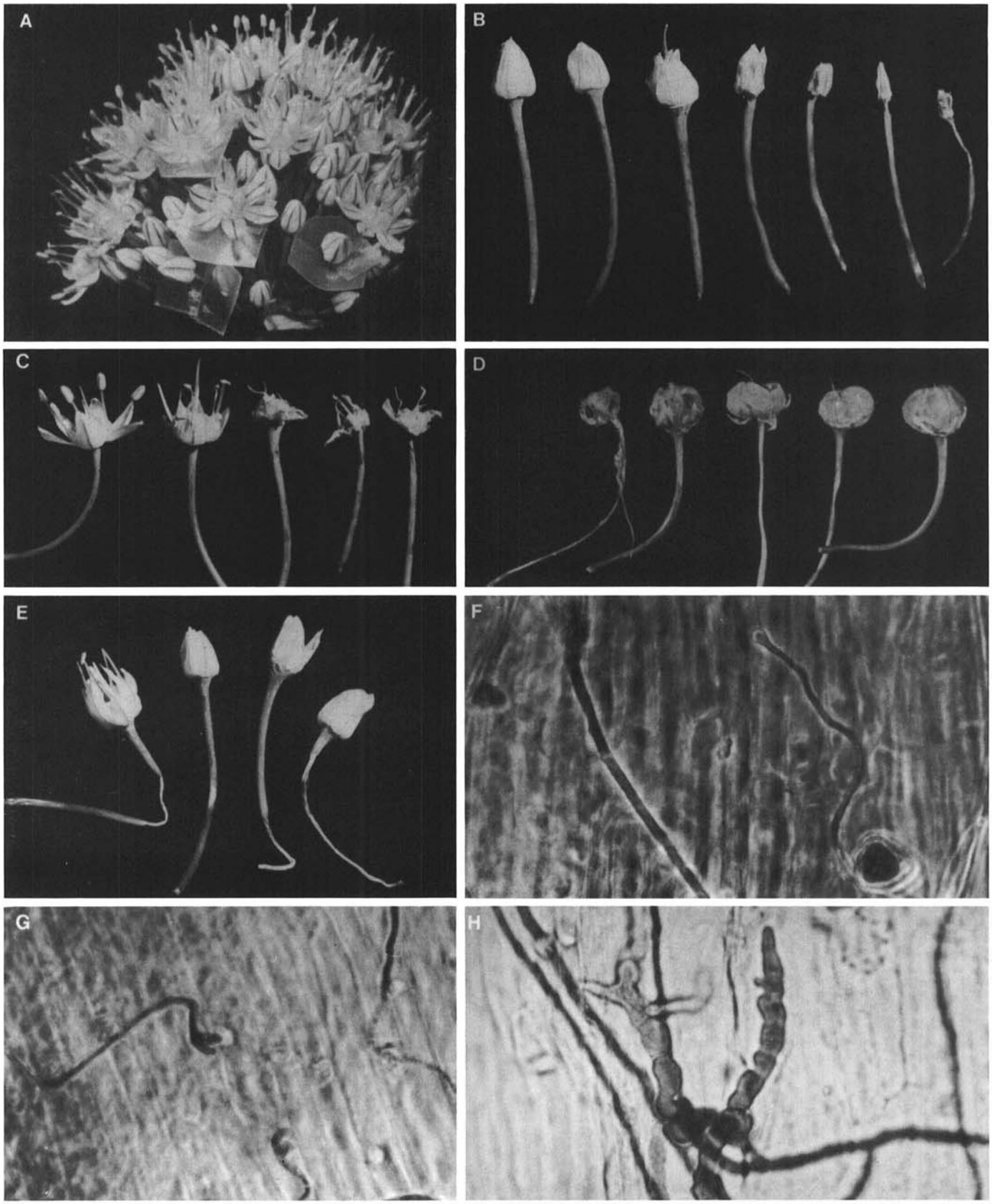

Fig. 1. Inoculation of florets on onion umbels with *Botrytis cinerea* in the dew chamber. Symptoms and infection structures were similar after inoculation with *B. squamosa* or *B. allii*. **A,** Umbel on which florets were marked with Parafilm collars (before inoculation). **B–D,** Sequences of symptom development. **B,** Unopened florets. **C,** Opened florets. **D,** Immature seed capsules. **E,** Pedicels girdled by *B. cinerea*. **F–H,** Infection structures of *B. cinerea* on adaxial petal surfaces. **F,** Single-lobed appressorium. **G,** Bilobed appressorium. **H,** Infection hyphae below a single-lobed appressorium.

All isolates of *B. squamosa* blighted greater percentages of florets than any of the isolates of *B. cinerea* or *B. allii* on umbels at stages 2 and 4 (Fig. 2A to C). There was a trend for isolates of *B. allii* to blight greater percentages of florets than isolates of *B. cinerea* when stage 2 umbels were inoculated. There also was a trend for isolates of *B. cinerea* to blight greater percentages of florets than isolates of *B. allii* when inoculated onto stage 4 umbels. When blighted florets were surface-disinfested and plated on acidified PDA, only the pathogen and an occasional saprophytic fungus were isolated.

Inoculum concentration. The percentages of blighted florets on umbels at stages 2 and 4 increased significantly as inoculum concentration of either *B. squamosa*, *B. cinerea*, or *B. allii* was increased (Fig. 3A and B). For umbels at both these stages, the regression models that best fit the data consisted of two lines with significantly different slopes and *Y*-intercepts. For each model, one line represented *B. squamosa* and the other line *B. cinerea* and *B. allii*. The slopes and intercepts of the separate lines representing either *B. cinerea* or *B. allii* were not significantly different.

The regression models indicated that *B. squamosa* blighted significantly higher percentages of florets than *B. cinerea* or *B. allii* over the range of inoculum concentrations tested (Fig. 3A and B). This higher percentage occurred when the pathogens were inoculated onto umbels at stages 2 and 4. In addition, *B. squamosa* was able to blight substantially greater percentages of florets at lower inoculum concentrations than were *B. cinerea* or *B. allii*. On stage 2 umbels, there was a trend (nonsignificant) for *B. allii* to blight a higher percentage of florets than *B. cinerea*. On stage 4 umbels, there was a trend (nonsignificant) for *B. cinerea* to blight higher percentages of florets than *B. allii*. Both of these trends occurred at the four inoculum concentrations tested.

Susceptibility of onion cultivars. There were no significant differences among the percentages of florets blighted on cultivars Early Yellow Globe, Early Yellow Medium, Italian Red, and Ontario M inoculated with either *B. squamosa*, *B. cinerea*, or *B. allii* (Fig. 2D). There also were no significant differences in the percentages of florets blighted on cultivars Early Yellow Globe and Sentinel inoculated with any one of the three pathogens.

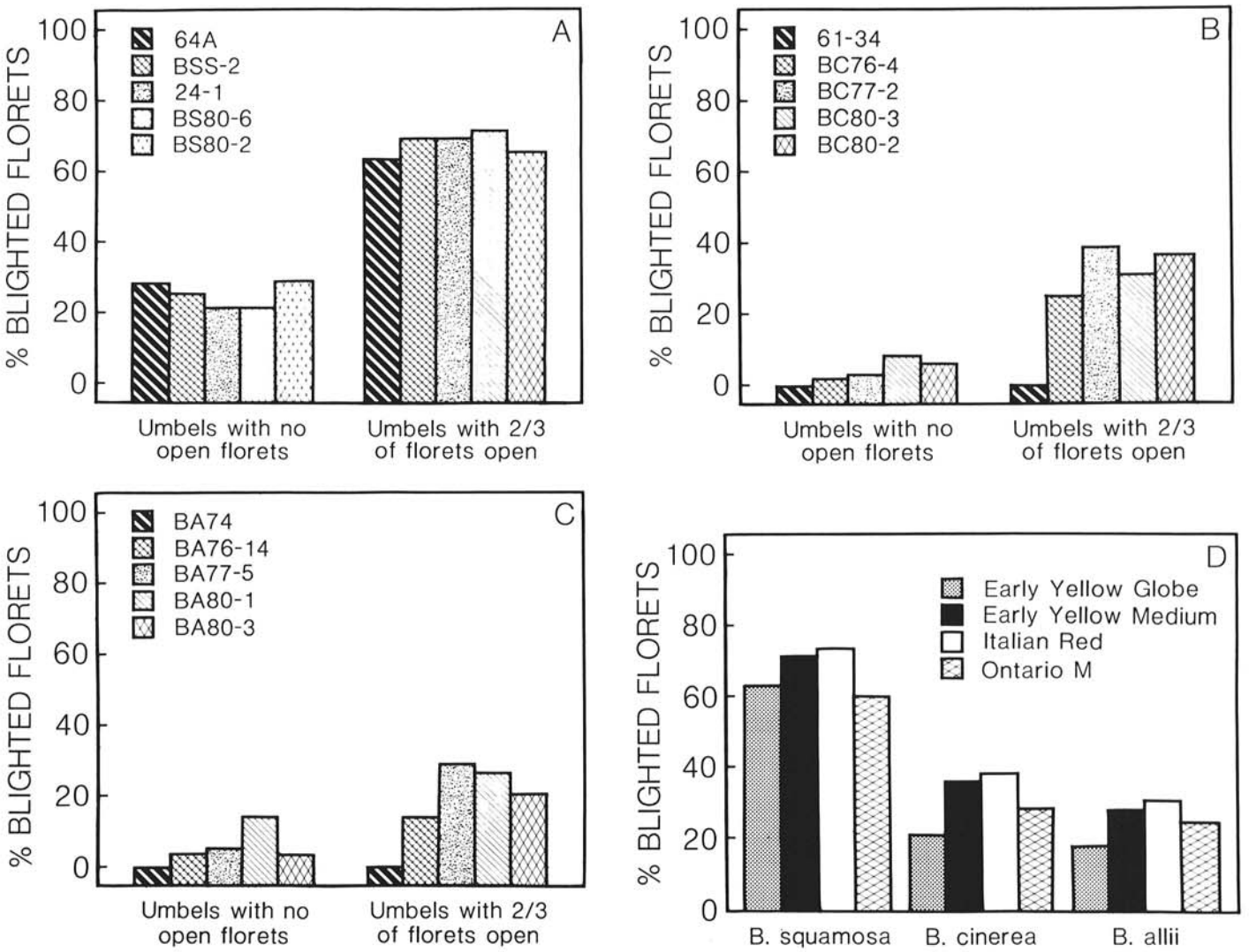

Fig. 2. The percentages of onion florets blighted by *Botrytis squamosa*, *B. cinerea*, and *B. allii* in the dew chamber. **A–C,** Suspensions of conidia (30,000 conidia per milliliter) of five isolates of each *Botrytis* species were atomized onto umbels (five replicates) with all florets unopened (stage 2 umbels) and umbels with approximately two-thirds of the florets opened (stage 4 umbels). **A,** Isolates of *B. squamosa*. The salient statistics were: for stage 2 umbels—MS (factor) = 51.1, MS (error) = 18.0, nonsignificant *F* statistic ($P = 0.05$); for stage 4 umbels—MS (factor) = 37.6, MS (error) = 18.5, nonsignificant *F* statistic ($P = 0.05$). **B,** Isolates of *B. cinerea*. The salient statistics were: for stage 2 umbels—MS (factor) = 33.95, MS (error) = 3.52, LSD = 2.8% ($P = 0.05$); for stage 4 umbels—MS (factor) = 956.1, MS (error) = 18.6, LSD = 6.5% ($P = 0.05$). **C,** Isolates of *B. allii*. The salient statistics were: for stage 2 umbels—MS (factor) = 105.2, MS (error) = 3.72, LSD = 2.9% ($P = 0.05$); for stage 4 umbels—MS (factor) = 527.14, MS (error) = 10.1, LSD = 4.8% ($P = 0.05$). **D,** The percentages of florets blighted on umbels of different onion cultivars by a single isolate of either *B. squamosa*, *B. cinerea*, or *B. allii*. Suspensions of conidia (30,000 conidia per milliliter) were atomized onto stage 4 umbels (four replicates). Early Yellow Globe, Early Yellow Medium, and Italian Red are homegrown, open-pollinated cultivars. Ontario M (Asgrow Seed Co., Kalamazoo, MI) is a hybrid cultivar. Salient statistics were: for *B. squamosa*—MS (factor) = 122.0, MS (error) = 102.0; for *B. cinerea*—MS (factor) = 127.4, MS (error) = 55.0; for *B. allii*—MS (factor) = 187.5, MS (error) = 72.5. *F* statistic values for the three *Botrytis* species were nonsignificant ($P = 0.05$).

Susceptibility of umbels at the five development stages. *B. squamosa*, *B. cinerea*, and *B. allii* were all pathogenic individually to umbels at stages 2–5, but *B. byssoidea* was not pathogenic to umbels at any development stage (Fig. 3C). *B. squamosa* was the only pathogen to blight florets on umbels at stage 1 via infection of the spathe. Taking into account the percentages of florets blighted by each *Botrytis* species on umbels at stages 1–5, *B. squamosa* blighted significantly more florets than *B. cinerea* or *B. allii*, and *B. cinerea* blighted significantly more florets than *B. allii* (Fig. 3C, Table 1). There was a trend for *B. allii* to blight more florets on umbels at stage 2 than *B. cinerea* and for *B. cinerea* to blight more florets than *B. allii* on umbels at stages 3–5.

Each of the pathogens blighted greater percentages of florets as the proportion of open to unopen florets on umbels at the time of inoculation was increased (Fig. 3C). Differences between means of the percentages of blighted florets for umbels at stages 1–5 usually were significant.

Susceptibility of unopen florets, open florets, and immature seed capsules. *B. squamosa*, *B. cinerea*, and *B. allii* each blighted a significantly lower percentage of unopen florets compared to open

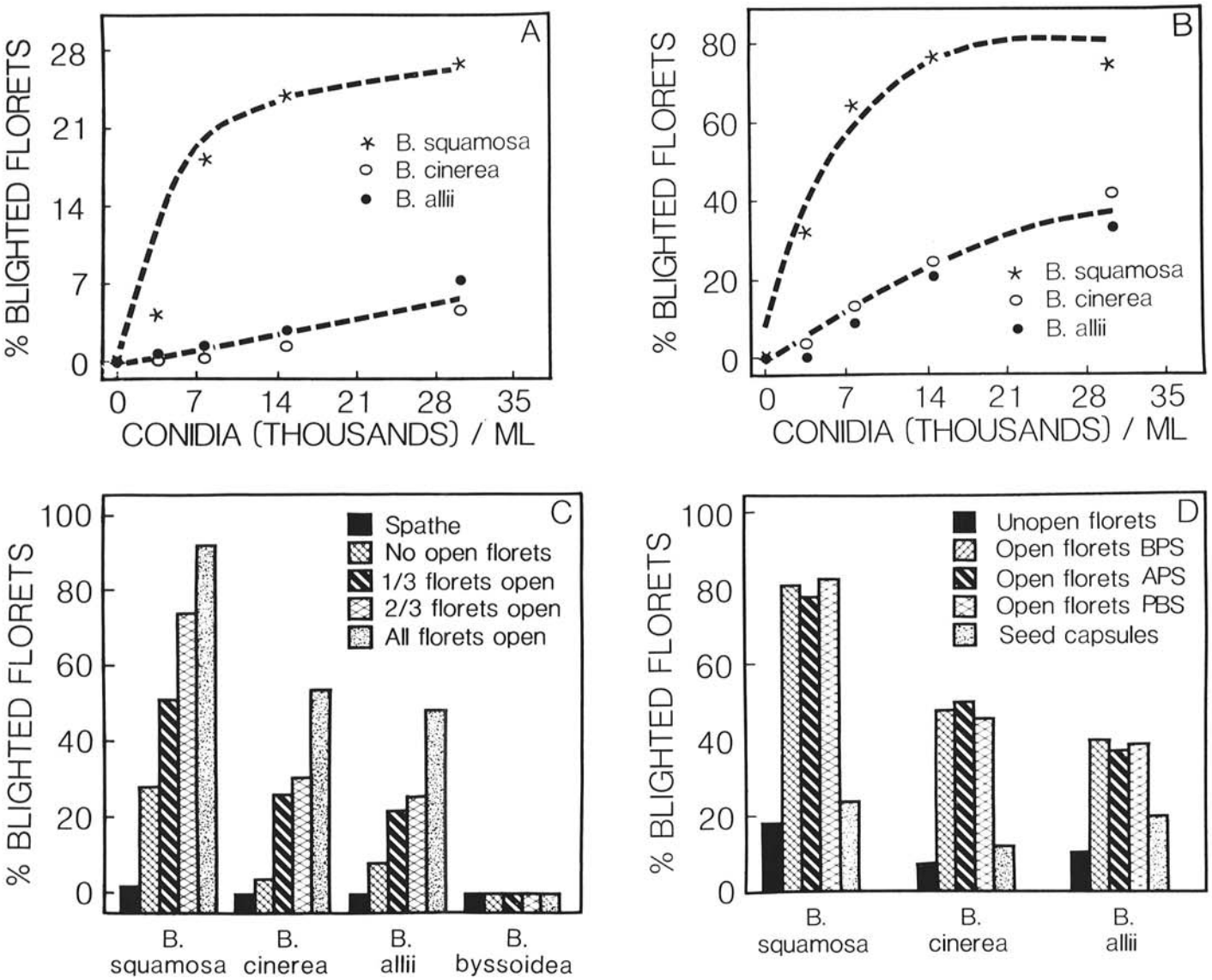

Fig. 3. The relationships of inoculum concentration, onion umbel development stage, and floret development stage to the percentages of florets blighted by *Botrytis* species in the dew chamber. **A and B,** The percentages of florets blighted by *B. squamosa*, *B. cinerea*, and *B. allii* at different inoculum concentrations on umbels with all florets unopened (stage 2 umbels) and on umbels with approximately two-thirds of the florets opened (stage 4 umbels). Suspensions of conidia at five concentrations (0, 3,750, 7,500, 15,000, and 30,000 conidia per milliliter) were atomized onto umbels. Dotted lines are regression lines with significantly different ($P = 0.01$) slopes and intercepts. **A,** Stage 2 umbels, $R^2 = 84.5$. **B,** Stage 4 umbels, $R^2 = 87.6$. **C,** The percentages of florets blighted on umbels at different development stages (stages 1–5) by either *B. squamosa*, *B. cinerea*, *B. allii*, or *B. byssoidea*. Suspensions of conidia (30,000 conidia per milliliter) were atomized onto umbels covered by the spathe (stage 1), open umbels with all florets unopened (stage 2), opened umbels with approximately one-third of the florets opened (stage 3), opened umbels with approximately two-thirds of the florets opened (stage 4), and opened umbels with all the florets opened (stage 5). The salient statistics were MS (model) = 3,850.0, MS (error) = 11.15, 19, and 80 df, respectively. The amounts of blight on umbels at each development stage caused by a particular *Botrytis* species were compared using Fisher's Protected LSD Test ($P = 0.05$, LSD = 4.3%). The combined amounts of blight on umbels at all of the development stages caused by each *Botrytis* species were compared by using statistical contrasts (Table 1). **D,** The percentages of unopened florets, opened florets (three stages), and immature seed capsules blighted by *B. squamosa*, *B. cinerea*, and *B. allii*. Unopened florets, opened florets before pollen shed (BPS), opened florets after pollen shed (APS), and opened florets as petals began to senesce (ABS) were marked with Parafilm collars on umbels with the majority of florets opened. Immature seed capsules were marked on umbels consisting entirely of immature seed capsules. Suspensions of conidia (30,000 conidia per milliliter) were atomized onto umbels of onion cultivar Sentinel (Joseph Harris Co. Inc., Rochester, NY) and blight was assessed only on marked florets. The salient statistics were MS (model) = 61.468, MS (error) = 1.289, 14 and 135 df, respectively. The percentages of unopened florets, opened florets, and immature seed capsules blighted by a single *Botrytis* species were compared by using Fisher's Protected LSD Test ($P = 0.05$, LSD = 15%). Other comparisons were made by using statistical contrasts (Table 2).

florets (Fig. 3D). There were no significant differences in the percentages of blighted florets among open florets before pollen shed, after pollen shed, or as petals began senescence. There were significantly lower percentages of immature seed capsules blighted by each *Botrytis* species than there were for open florets.

B. squamosa blighted a significantly higher percentage of unopen florets than either *B. cinerea* or *B. allii*. *B. allii* did not blight significantly more unopen florets than *B. cinerea* (Fig. 3D, Table 2). There was a trend for *B. allii* to blight more unopen florets than *B. cinerea*. *B. squamosa* blighted significantly more open florets than *B. cinerea*, which in turn blighted significantly more open florets than *B. allii*. *B. squamosa* also blighted significantly more immature seed capsules than either *B. cinerea* or *B. allii*. There was a trend for *B. allii* to blight a higher percentage of immature seed capsules than *B. cinerea*.

Dual inoculations. Dual inoculation of umbels with *B. squamosa* and either *B. cinerea* or *B. allii* simultaneously, 48 hr earlier, or 48 hr later did not cause blight in a significantly higher percentage of florets than did inoculation with the pathogens individually (Tables 3 and 4). When inoculation was with either *B. cinerea* or *B. allii* individually, or with *B. cinerea* 48 hr before or after *B. allii*, there were no significant differences in the percentages of blighted florets on umbels (Table 5). When inoculation was with *B. allii* and *B. cinerea* simultaneously, the percentages of blighted florets on

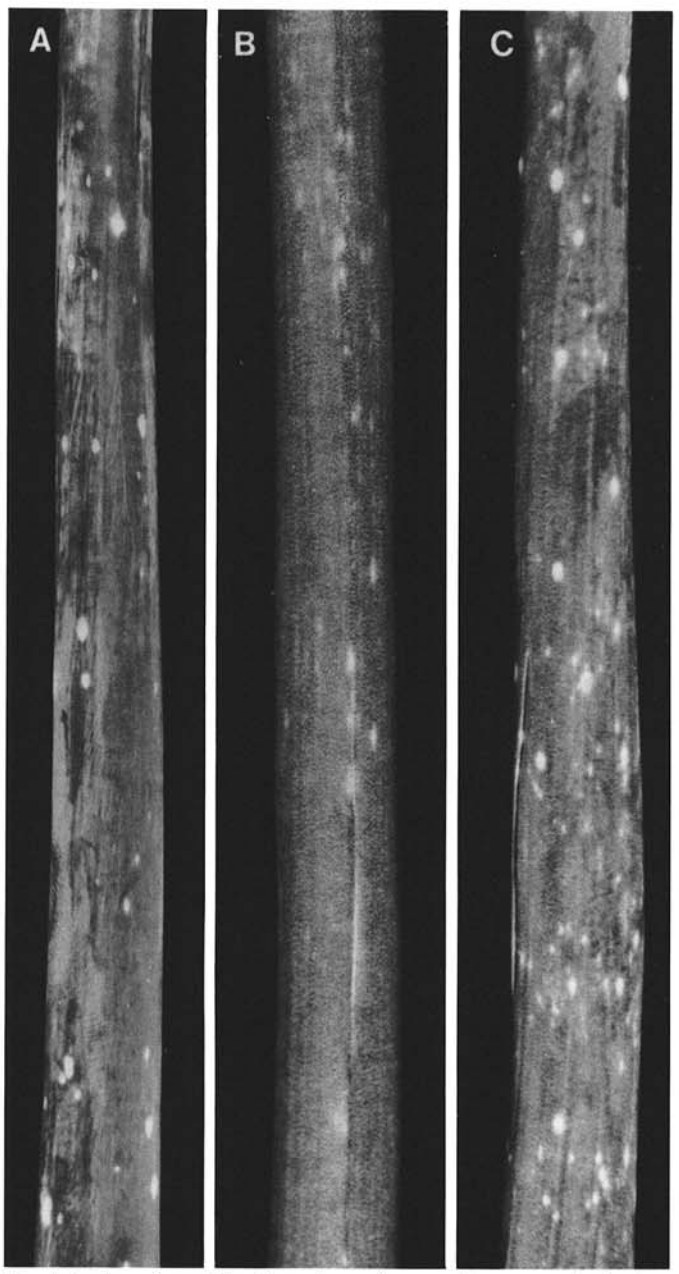

Fig. 4. Lesions formed by *Botrytis* species on onion scapes in the dew chamber: **A,** *B. squamosa*; **B,** *B. cinerea*; and **C,** *B. allii*.

TABLE 1. Statistical contrasts of the percentages of blighted florets resulting from inoculation of onion umbels at different development stages with four *Botrytis* species[a]

Contrast[b]	*T*-value[c]	Significance level (*P*)
B. byssoidea compared to the other species	40.03	0.0001
B. squamosa compared to the other species	33.94	0.0001
B. cinerea compared to *B. allii*	2.03	0.0454

[a]Statistical contrasts were performed by using the "estimate" option of a Statistical Analysis Systems general linear model program (SAS Institute, Cary, NC). The data used to develop these contrasts are illustrated in Fig. 3C.
[b]Contrasts are for the combined blighting on umbels at all of the five development stages by each *Botrytis* species.
[c]The *T*-value is the estimate divided by the standard error.

TABLE 2. Statistical contrasts of the percentages of florets at a particular onion umbel development stage blighted by either *B. squamosa*, *B. cinerea*, or *B. allii*[a]

Contrast[b]	*T*-value[c]	Significance level (*P*)
BS 1 versus BC 1 + BA 1	2.05	0.0427
BC 1 versus BA 1	0.39	0.6944
BS 2 + BS 3 + BS 4 versus BS 2 + BC 3 + BC 4	14.04	0.0001
BC 2 + BC 3 + BC 4 versus BA 2 + BA 3 + BA 4	2.71	0.0075
BS 5 versus BC 5 + BA 5	6.37	0.0010
BC 5 versus BA 5	1.58	0.1176

[a]Statistical contrasts performed using the "estimate" option of a Statistical Analysis Systems general linear model program (SAS Institute, Cary, NC). Data used are illustrated in Fig. 3D.
[b]BS, BC, and BA represent florets or immature seed capsules inoculated with *B. squamosa*, *B. cinerea*, and *B. allii*, respectively. The five floret development stages are represented by 1, 2, 3, 4, and 5, respectively. The notation "BS 1 versus BC 1 + BA 1" indicates that a comparison of the percentages of blighted florets was made between unopen florets inoculated with *B. squamosa* and unopen florets inoculated with *B. cinerea* and *B. allii*.
[c]The *T*-value is the estimate divided by the standard error.

TABLE 3. Percentages of blighted florets resulting from inoculation of onion umbels with *Botrytis squamosa* and *B. allii* and reisolation of these pathogens[a]

Inoculum[b]	Blighted florets[c] (%)	Florets from which the pathogens were reisolated (%)	
		B. squamosa	*B. allii*
B. squamosa alone	93 a	87	0
B. allii alone	33 b	0	100
B. squamosa and *B. allii* together	80 a	0	100
B. allii 48 hr after *B. squamosa*	81 a	53	40
B. squamosa 48 hr after *B. allii*	85 a	40	50

[a]Onion cultivar was homegrown Early Yellow Globe.
[b]Umbels with all florets open were inoculated with conidia applied in water suspensions containing 30,000 conidia per milliliter.
[c]Disease was assessed 10 days after inoculation. Data are the mean percentages for four replicates (one umbel per replicate). No blight was observed on uninoculated controls and no fungi were isolated from uninoculated florets. Data followed by the same letter are not significantly different according to Fisher's Protected LSD Test ($P = 0.05$).

inoculated umbels was significantly higher than for any other treatment.

In each of the experiments when inoculation was with either *B. squamosa*, *B. cinerea*, or *B. allii* individually, only the introduced pathogen was reisolated, indicating freedom from cross contamination in the dew chamber (Tables 3–5). When inoculation was with *B. squamosa* and *B. allii* simultaneously, only *B. allii* was reisolated. When inoculation was with *B. squamosa* and *B. cinerea* simultaneously, the latter was reisolated from 83% of the blighted florets and *B. squamosa* was reisolated from 7%. When inoculation was with two of the *Botrytis* species 48 hr apart, the one introduced first usually was reisolated. However, when inoculation with *B. allii* was 48 hr after *B. cinerea*, *B. allii* was isolated more frequently from blighted florets than was *B. cinerea*.

Symptoms and signs in controlled inoculation. There were no differences in the symptoms on florets blighted by *B. squamosa*, *B. cinerea*, or *B. allii* in the inoculation experiments. However, symptoms did differ among unopen florets, open florets, and immature seed capsules inoculated with any of the three pathogens (Fig. 1B to D). During early symptom development (3–4 days), infected open florets did not appear appreciably different from open florets that were senescing normally. Petals, stamens, and styles gradually withered on both infected and senescing florets. On the infected florets, however, the shriveling occurred over a shorter period (3–4 days) than required for normal senescence of noninfected florets of the same age (up to 10 days). Five to 6 days after inoculation, the first symptoms of necrosis of the ovary were observed on infected florets. Necrosis of the ovary first occurred as irregular lesions either near bases of petals, base of the style, or on other areas of the ovary. By 10 days after inoculation, the ovaries of infected florets were totally necrotic and necrosis was beginning to progress down the pedicels. Infected florets only appeared (to the unaided eye) appreciably different from noninfected florets when the ovaries became necrotic.

Symptom development on florets unopened at the time of inoculation was dependent on the age of the floret. If infection occurred on an unopen floret early in its development, subsequent necrosis was usually sufficient to prevent opening, and the unopen floret generally aborted. If infection occurred 1–2 days before a floret opened, the macroscopic symptoms were limited to withered petals and/or turgid petals on which different amounts of the surface area were either water-soaked or translucent. Pistils of these florets infrequently became necrotic.

Hyphae of either *B. squamosa*, *B. cinerea*, or *B. allii* usually were observed ramifying both over the surfaces and intercellularly within the petals of inoculated unopen florets and within the petals, stamens, and styles of inoculated open florets. Each of the three *Botrytis* species penetrated the host tissue by means of a single or bilobed appressorium which usually formed over anticlinal cell wall junctures (Fig. 1F and G). Intracellular infection hyphae formed below appressoria (Fig. 1H) and intercellular hyphae formed from these infection hyphae. Frequently, the petals and/or stamens and styles of florets that appeared to be senescing normally were observed to be infected with one of the three pathogens.

On immature seed capsules, irregular lesions were first observed 5–6 days after inoculation. These lesions commonly occurred near attachments of the styles and petals but also occurred on other areas of the capsule. The lesions usually expanded until the entire capsule and the enclosed seeds were destroyed. However, on capsules which were relatively mature at inoculation, necrosis of the capsule usually was limited to irregular lesions and the seeds appeared to be unaffected.

In all inoculation experiments, 8–12% of the florets or seed capsules blighted by either *B. squamosa*, *B. cinerea*, or *B. allii* were aborted due to infection and girdling of the pedicels (Fig. 1E). When umbels were inoculated while the majority of florets were open (stages 4 and 5), 8–10% of the florets that were blighted had girdled pedicels. When umbels were inoculated while the majority of florets were unopen (stages 2 and 3), or while the umbel consisted entirely of immature seed capsules, 10–12% of the blighted florets or immature seed capsules had girdled pedicels. The slightly lower incidence of pedicel girdling on umbels with the majority of florets open compared to umbels at other stages of development is believed to be due to the petals of the open florets obstructing access of inoculum to the pedicels.

On scapes, *B. squamosa*, *B. cinerea*, and *B. allii* each produced elliptical lesions (Fig. 4A to C) which began as water-soaked areas with central pinpoint areas of necrosis. Lesions of *B. squamosa* formed more quickly (2–3 days) than lesions of the other species (3–4 days) and were larger. Lesions produced by *B. allii* were larger than those produced by *B. cinerea*. Only lesions produced by *B. squamosa* and *B. cinerea* were observed to expand and girdle scapes. Girdling of scapes usually required 14–18 days. *B. squamosa*, *B. cinerea*, and *B. allii* each produced lesions on all areas of the scapes.

Development of signs of the pathogens on umbels or scapes depended on the conditions of incubation after inoculation. If umbels and scapes were placed in the walk-in growth chamber at ambient humidity after incubation in the dew chamber, only *B. allii* sporulated on the necrotic surfaces. If umbels and scapes were first incubated in the dew chamber and then placed in the walk-in growth chamber, *B. squamosa* and *B. cinerea* would only sporulate if the umbels and scapes were returned to the dew chamber. Sclerotia of *B. squamosa* sometimes formed on scapes that had

TABLE 4. Percentages of blighted florets resulting from inoculation of onion umbels with *Botrytis squamosa* and *B. cinerea* and reisolation of these pathogens[a]

Inoculum[b]	Blighted florets[c] (%)	Florets from which the pathogens were reisolated (%)	
		B. squamosa	*B. cinerea*
B. squamosa alone	84 a	83	0
B. cinerea alone	45 b	0	100
B. squamosa and *B. cinerea* together	79 a	7	83
B. cinerea 48 hr after *B. squamosa*	87 a	53	23
B. squamosa 48 hr after *B. cinerea*	81 a	33	60

[a] Onion cultivar was homegrown Early Yellow Globe.
[b] Umbels with all florets open were inoculated with conidia applied in water suspensions containing 30,000 conidia per milliliter.
[c] Disease was assessed 10 days after inoculation. Data are the mean percentages for four replicates (one umbel per replicate). No blight was observed on uninoculated controls and no fungi were isolated from uninoculated florets. Data followed by the same letter are not significantly different according to Fisher's Protected LSD Test ($P = 0.05$).

TABLE 5. Percentage of blighted florets resulting from inoculation of onion umbels with *Botrytis cinerea* and *B. allii* and reisolation of these pathogens[a]

Inoculum[b]	Blighted florets[c] (%)	Florets from which the pathogens were reisolated (%)	
		B. cinerea	*B. allii*
B. cinerea alone	38 a	100	0
B. allii alone	58 a	0	100
B. cinerea and *B. allii* together	81 b	93	50
B. cinerea 48 hr after *B. allii*	53 a	0	100
B. allii 48 hr after *B. cinerea*	40 a	47	70

[a] Onion cultivar was homegrown Early Yellow Globe.
[b] Umbels with all florets open were inoculated with conidia applied in water suspensions containing 30,000 conidia per milliliter.
[c] Disease was assessed 10 days after inoculation. Data are the mean percentages for four replicates (one umbel per replicate). No blight was observed on uninoculated controls and no fungi were isolated from uninoculated florets. Data followed by the same letter are not significantly different according to Fisher's Protected LSD Test ($P = 0.05$).

been incubated in the dew chamber and then placed in the walk-in growth chamber for 2 wk or more.

DISCUSSION

B. squamosa, *B. cinerea*, and *B. allii* individually caused onion flower blight in controlled inoculations conducted during this study. A previous report indicated that when *B. cinerea*, *B. squamosa*, and *B. allii* were inoculated individually onto onion umbels under field conditions, seed yields were reduced by 97, 93, and 47%, respectively, due to blighting of florets and immature seed capsules (4). The relative virulence of the three *Botrytis* species in the present study was indicated by the amount of blighting of onion florets and immature seed capsules resulting from controlled inoculations. In the study of Ellerbrock and Lorbeer (4), virulence of these fungi was indicated by reductions in seed yield resulting from inoculations of onion umbels under field conditions, and the amount of blighting was not assessed. Ellerbrock and Lorbeer (4) reported that *B. cinerea* and *B. squamosa* were equally virulent, while *B. allii* was less virulent. In controlled inoculations conducted in the current study, *B. squamosa* was consistently more virulent than *B. cinerea* or *B. allii*, which were similar in virulence. Both studies indicated that *B. byssoidea*, a species of *Botrytis* commonly isolated from blighted onion florets, was nonpathogenic (4,12). The discrepancies between results of the study by Ellerbrock and Lorbeer (4) and those of the present study may have been due to a different relative virulence of the isolates used for inoculations and/or to the different conditions to which umbels were exposed during and after inoculation. Because the inoculations of Ellerbrock and Lorbeer (4) were conducted in the field, the possibility of interactions of the experimental fungi with other organisms cannot be overlooked. In this study, however, the percentage of blighted florets resulting from any dual inoculation with paired pathogens was not higher than the additive amount of blight caused by inoculation with each pathogen separately.

In the present study, isolates of *B. squamosa* were all highly virulent and isolates of *B. byssoidea* were all nonpathogenic. Isolates of *B. cinerea* and *B. allii* differed in virulence, but no isolate of either species was as virulent as any isolate of *B. squamosa*. These results were comparable with data from the field.

Hancock and Lorbeer (5) compared lesion production as a function of conidial concentration for *B. squamosa*, *B. cinerea*, and *B. allii* on onion leaves. They reported that *B. squamosa* caused a greater number of lesions than either *B. cinerea* or *B. allii* at inoculum concentrations from 10^5 to 10^6 conidia per milliliter. In the present study, *B. squamosa* consistently blighted higher percentages of florets than *B. cinerea* or *B. allii* at inoculum concentrations of 3,750 to 30,000 conidia per milliliter. Hancock and Lorbeer (5) reported that *B. cinerea* consistently produced more lesions than *B. allii* at all concentrations of conidia tested. However, no significant difference was demonstrated between the percentages of florets blighted by either *B. cinerea* or *B. allii* at any conidial concentration that was tested.

No difference in susceptibility of onion cultivars to Botrytis leaf blight caused by *B. squamosa* has been reported. In the present study, there was no significant difference in the susceptibility of umbels of three onion cultivars commonly grown for open-pollinated homegrown seed production in Orange County or of two hybrid cultivars to either *B. squamosa*, *B. cinerea*, or *B. allii*.

Although *B. cinerea* is pathogenic on many flowers (2,6,7,9), the onion umbel is the only inflorescence on which *B. squamosa* and *B. allii* have been reported as pathogenic. In the current study, onion umbels and florets at different development stages differed in susceptibility to *B. squamosa*, *B. cinerea*, and *B. allii*. Varying susceptibility of inflorescences at different development stages to *B. cinerea* has been reported for other crops, including strawberry (8) and grape (9), on which the pathogen prefers damaged or senescent tissues. McClellan and Hewitt (9) reported that grape flowers at prebloom were less susceptible to infection by *B. cinerea* than were flowers more advanced in development. In the study reported here, onion umbels at the onset of the prebloom development stage (the umbel covered with the spathe) were less susceptible than those at later stages (open umbels) to blighting by *B. squamosa*. Inoculation with *B. cinerea* or with *B. allii* did not lead to blighting. Differences in susceptibility of open umbels were due to differences in the susceptibility of florets at different development stages. As the number of open florets on umbels increased, more florets were blighted by each of the three *Botrytis* species. An investigation of the susceptibility of florets at different development stages indicated that unopen florets were significantly less susceptible than open florets to *B. squamosa*, *B. cinerea*, and *B. allii* (individual inoculations). This difference in susceptibility is in concert with the report of Jarvis (7), which indicated that unopen strawberry flowers are less susceptible than open flowers to *B. cinerea*. Senescent strawberry flowers are less susceptible to *B. cinerea* than open flowers before senescence commences. In contrast, in the current study, onion florets that had begun to senesce were as susceptible to blighting by either *B. cinerea*, *B. squamosa*, or *B. allii* as were open florets before senescence. *B. cinerea* has been reported (7–9,11) to infect mature and/or senescent floral parts and later to infect the fruit of grape, strawberry, raspberry (*Rubus idaeus* L.), almond (*Prunus amygdalus* Batsch), apricot (*P. armeniaca* L.), and plum (*P. mume* Sieb. & Zucci). Because the petals, stamens, and styles remain attached to immature onion seed capsules, it seemed possible that one or more of the pathogens might colonize senescent floret parts and later invade the seed capsule. In accordance with that idea, necrosis of seed capsules frequently was observed to begin near the attachment of petals and styles. Direct infection of capsules by *B. squamosa*, *B. cinerea*, and *B. allii* also was observed.

The difference between the susceptibilities of the five umbel development stages tested for infection by *B. squamosa*, *B. cinerea*, and *B. allii* has a bearing on the timing of fungicide sprays to control onion flower blight. Due to the low susceptibility of umbels covered by the spathe, fungicides would need only be applied to umbels at this stage if disease pressure was high. As umbels in seed fields begin to open and florets begin to bloom, the need for fungicide application would increase if environmental conditions were favorable for disease development. As the number of open florets in seed fields decreased and the number of seed capsules increased, the need for fungicide sprays would decrease unless disease pressure was high.

In the current study, only florets with obvious onion flower blight symptoms (necrosis of the pistil or seed capsule) were assessed as blighted. Observations indicated that many florets were infected but did not show obvious symptoms (symptoms different from the appearance of normally senescing florets). It was not determined how many infections or which lesion locations were most likely to cause blighting of a floret. It was not determined if infections that did not result in blighting of the floret could become latent and the fungus later colonize developing seed capsules. It also was not determined if the greater virulence of *B. squamosa*, compared to *B. cinerea* or *B. allii*, was due to the occurrence of a greater number of infections or to greater aggressiveness of *B. squamosa* after infection.

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