Comparative Nutrient Dependency of Botrytis squamosa and B. cinerea for Germination of Conidia and Pathogenicity on Onion Leaves

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ABSTRACT

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Germination of conidia and germ tube length of isolates of *Botrytis squamosa* were similar in water and nutrient solution. Spraying conidia in water onto leaves resulted in production of expanding lesions. The frequency of lesions was increased by addition of nutrients to the inoculum. Germination of conidia of all isolates of *B. cinerea* was significantly lower in water than in nutrient solution. Germ tube elongation in water ceased almost immediately upon emergence of the germ tube from the conidium. Spraying onion leaves with conidia suspended in water failed to produce lesions, but addition of an exogenous nutrient source resulted in formation of expanding lesions. Nutrient dependency of conidia of *B. cinerea,* but not of *B. squamosa,* increased with increased age of dry-harvested conidia. Combining the two species during incubation had no effect on conidial germination. Leaching conidia of either species with water inhibited germination, but leaching with nutrient solution did not. Conidial germination and lesion formation (numbers) by both species were increased by diffusate from the onion-leaf lacuna, filtrate from cattail pollen, or by Czapek-Dox broth amended with yeast extract. Glucose (1%) was a weak stimulant for both germ tube elongation and lesion formation.

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

Botrytis squamosa Walker produces expanding lesions (spots extend through the leaf and chlorotic, water-soaked halos expand out from the spot) followed by blighting of onion (*Allium cepa* L.) leaves. *Botrytis cinerea* Pers. forms superficial flecks which do not expand on onion leaves (17). When conidia in water suspension are injected into the lacuna of an onion leaf, both *B. squamosa* and *B. cinerea* cause substantial blighting. Under the latter conditions, both species produce pectolytic enzymes which alone can mimic the symptoms of Botrytis leaf blight of onion (18).

Conidial germination and pathogenesis by *B. cinerea* are known to be stimulated significantly on other suscepts by nutrients (1, 9, 20, 22, 24, 33). Mycelium of *B. cinerea* did not grow in water on onion leaves following emergence of the germ tube, but when nutrients were added, superficial growth was extensive and penetration ensued (11). Although *B. squamosa* did not grow extensively on the onion leaf surface without exogenous nutrients, it responded tropically to the leaf surface, penetrated rapidly, and produced expanding lesions (11).

This study was initiated to determine whether differing nutrient dependency of *B. cinerea* and *B. squamosa* for conidial germination and lesion formation accounted for differences in pathogenicity of the two species on onion leaves.

MATERIALS AND METHODS

General.—Cultures of *Botrytis squamosa* and *B. cinerea* were maintained by monoconidial transfers to slants of a complete medium (Difco Czapek-Dox broth, 35 gm; Difco yeast extract, 2.5 gm; Difco malt extract, 7.5 gm; hydrolyzed casein, 250 mg; sodium nucleate, 10 mg; trace element solution, 1 ml; agar, 15 gm; and distilled water, 1,000 ml). The trace element stock solution consisted of: $Fe(NO_3)_3 \cdot 9H_2O$, 723.5 mg; $ZnSO_4 \cdot 4\ H_2O$, 203.0 mg; H_3BO_3, 2.0 mg; H_3MoO_3, 2.0 mg; $CuSO_4$, 2.0 mg; and distilled water, 1,000 ml (2). The slant cultures were incubated at 21 C under fluorescent light (Sylvania F20 T12-CW, 12-hour photoperiod).

Conidia, both for incubation tests in vitro and for inoculations, were produced either on the complete medium or onion-leaf-straw agar (dry onion leaf straw was placed in 9-cm diameter petri plates, 1.5% water agar added, and the plates were autoclaved). Conidia of *B. squamosa* or *B. cinerea,* grown for 2-6 weeks on the complete-medium slants, were suspended in 10 ml of sterile distilled water. Aliquots (0.3 ml) of the conidial suspensions were transferred aseptically to complete-medium plates and spread uniformly over the agar

surface with a glass spreader. Conidial suspension (1 ml) was pipetted over the surface of onion-leaf-straw agar. Plates were incubated at 21 C under fluorescent and near-ultraviolet light (12-hour photoperiod). On complete medium, sporulation of *B. cinerea* and *B. squamosa* began after 4 days and 6 days of incubation, respectively. Conidia were harvested after 5-6 days and 7-8 days incubation for *B. cinerea* and *B. squamosa,* respectively. On onion-leaf-straw agar both species began sporulating after 3 or 4 days of incubation and conidia were harvested after 5-6 days by aspiration into sterile flasks (250-ml Erlenmeyer) containing 30 ml of sterile glass-distilled water, thus avoiding nutrient contamination from the medium.

Isolates 74-67, 74-71, 74-107, 75-2, and 75-3 of *B. squamosa* were obtained by L. A. Ellerbrock from onions grown in New York, and isolate 413 was obtained from an onion grown in Texas. Isolates 70-35, 70-37, 71-1, 71-2, 71-4, 71-5, 72-2, 72-7, 72-11, 72-13, 72-17, and 72-19 of *B. cinerea* were obtained by C. A. Clark from onions grown in New York. Isolates 70-8 and 70-11 were obtained by C. A. Clark in New York from sweet cherry and strawberry, respectively. Isolates 70-29 from *Nicotiana rustica* and 70-25 were supplied from Scotland by W. R. Jarvis. Isolate 70-33 was supplied from England by T. F. Preece. Unless otherwise specified, isolate 61-34 of *B. cinerea* obtained from onions grown in New York and mutant isolate 64a (2) of *B. squamosa* were used.

Conidia were germinated in 0.6 ml of liquid in glass dishes (22-mm diameter, 5-mm deep) made by cutting off the bottoms of shell vials. During incubation, the dishes were placed in sterile petri dishes containing a piece of filter paper moistened with 5% glycerine (to reduce condensation). To determine the time required for germination, conidia were incubated in 50 ml of sterile glass-distilled water or Czapek-Dox broth (50%) plus yeast extract (0.05%) in 250-ml Erlenmeyer flasks on platform shakers. Aliquots were removed at different times and transferred to a drop of cotton blue in lactophenol on a microscope slide. Conidia were incubated for 12 hours in darkness at 21 C and then were killed and stained by adding one drop of cotton blue in lactophenol per incubation dish. Nutrient contamination was minimized by prior rinsing of all glassware in chromic acid cleaning solution. Czapek-Dox broth (50%) plus yeast extract (0.05%) was used as a standard nutrient source.

Two sources of plants were used for inoculation. In some cases 3- to 5-month-old plants (cultivar Elba Globe) which had been grown from seed were used. In other experiments, 2- to 4-week-old plants (cultivar Ontario L) grown from bulbs were used. Inoculated plants were incubated in a mist chamber at 21 C under fluorescent light (Sylvania F96 T12-WW-VHO, 12-hour photoperiod). Free moisture was supplied by applying mist for 15 seconds every 10 minutes. Two humidifiers (operating continuously and independently) maintained the free moisture on leaf surfaces. Plants were placed in the mist chamber 24 hours prior to inoculation. Each plant was inoculated by spraying it with 5-10 ml of conidial suspension containing one drop of Tween-20 per 100 ml of suspension. To obtain uniform lesion densities, conidial suspensions of *B. squamosa* were adjusted to either 30,000/ml in water or 3,000/ml in nutrient solution, whereas *B. cinerea* was adjusted to 30,000/ml in both. Numbers of lesions per leaf were counted 48 hours after inoculation.

Substrate.—Substrata compared for nutrient dependency of conidia were: complete medium, onion-leaf-straw agar, excised onion leaves, and Difco potato-dextrose agar (PDA). Excised onion leaves were surface-sterilized in 0.5% sodium hypochlorite for 5 minutes and rinsed in two changes of sterile distilled water. The leaves then were placed on wet filter paper in sterile petri dishes.

Effect of age of conidia on nutrient dependency.—Conidia of different ages were harvested from complete-medium cultures at the onset of sporulation (4 days of incubation for *B. cinerea* and 6 days for *B. squamosa*). The conidia (24-hours-old or less) were placed in dry, sterile 250-ml Erlenmeyer flasks which were sealed with aluminum foil and placed for different storage times under the conditions previously described for incubating cultures. The conidia which had been stored for different lengths of time were suspended in

TABLE 1. The effect of a nutrient solution[a] on germination (in vitro) of conidia and formation of lesions on onion leaves of *Botrytis squamosa* isolates

Isolate	Germination[b] (%)		Germ tube length (μm)		Lesion counts[c]	
	Water	Nutrient solution	Water	Nutrient solution	Water	Nutrient solution
74-71	99	97	96	117	...	...
74-107	99	99	92	165	...	...
74-67	99	98	50	129	135	911
75-2	99	98	80	157	...	...
413	99	98	85	112	132	483
64a	100	97	87	95	98	517
75-3	100	98	125	101	71	531

[a]Czapek-Dox broth (50%) plus yeast extract (0.05%).

[b]Differences were not significantly different ($P = 0.05$) in individual experiments. Numbers are the averages for two independent experiments (three replicates/experiment) after 12 hours of incubation.

[c]Number of lesions per leaf mathematically adjusted for differences in inoculum concentration (lesions per leaf with nutrients were multiplied by 10). Numbers are the averages for two independent experiments (four replicate plants per experiment). Differences between isolates were not significantly different ($P = 0.05$). In all cases differences between water and nutrients were significantly different ($P = 0.0$).

sterile glass-distilled water and incubated in the glass incubation dishes.

Nutrient sources.—Conidia of both species were incubated in vitro and inoculated onto onion leaves in 1% glucose, onion lacunar diffusate, or filtrate of cattail pollen (*Typhula* spp.). Cattail pollen was used because large quantities could be readily obtained. Onion lacunar diffusate was prepared by filling the lacuna of excised whole onion leaves (cultivar Elba Globe) with glass-distilled water; after holding for 1 hour at 20-25 C, the diffusate was collected. Cattail pollen was suspended (2×10^6 grains/ml) in distilled water and frozen for storage. Both preparations were filter-sterilized (Millipore, 0.22-μm pore size) prior to use.

Leaching.—Conidia of each species were collected on a Millipore filter. A second 0.22-μm pore-size Millipore

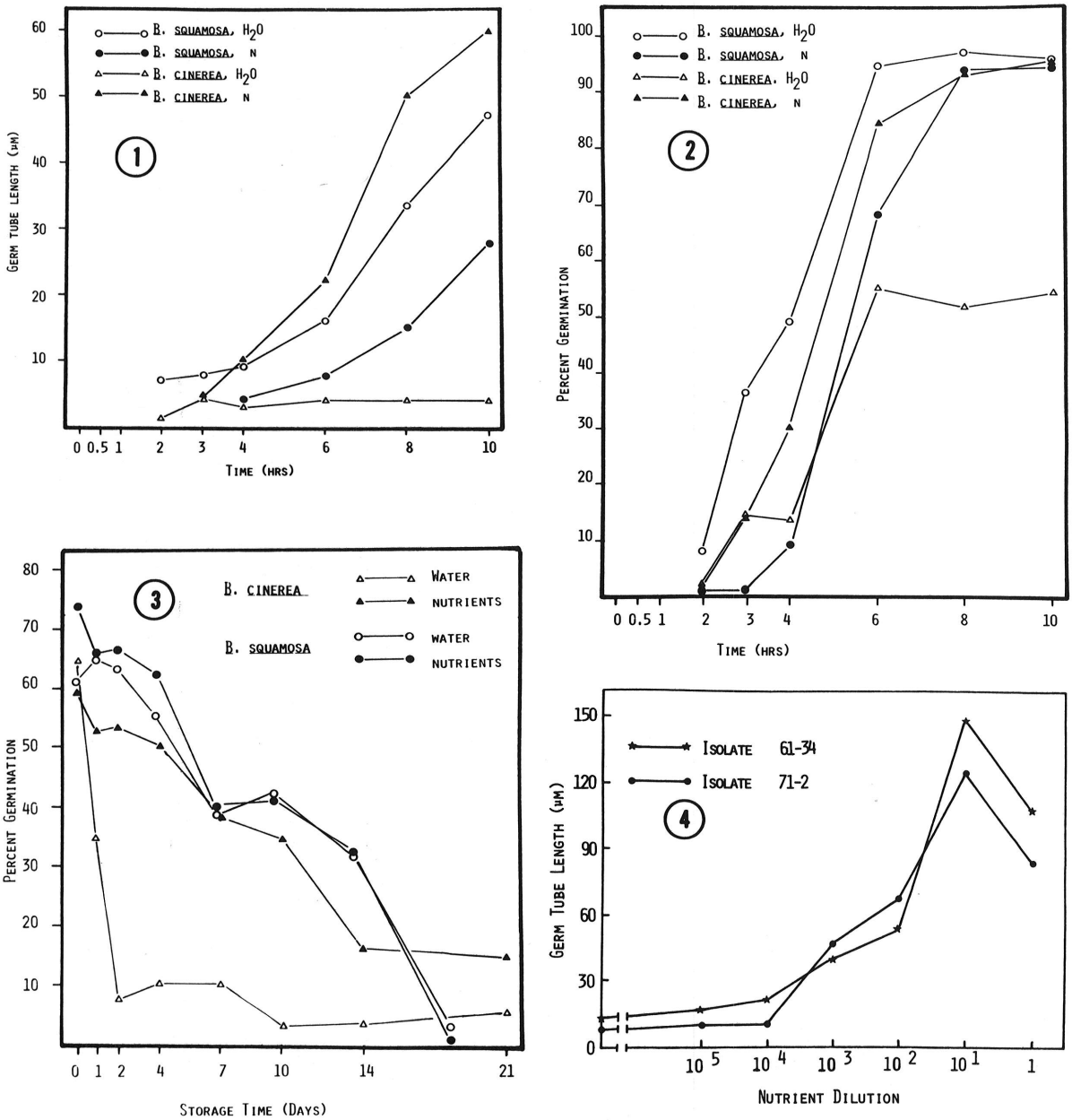

Fig. 1-4. 1) Germ tube elongation of conidia of *Botrytis cinerea* (isolate 61-34) and *B. squamosa* (isolate 64a) in water or Czapek-Dox broth (50%) plus yeast extract (0.05%) as related to time of incubation. **2)** Germination (%) of conidia of *Botrytis cinerea* (isolate 61-34) and *B. squamosa* (isolate 64a) in water or Czapek-Dox broth (50%) plus yeast extract (0.05%) as related to time of incubation. **3)** Germination (%) of conidia of *Botrytis squamosa* (isolate 64a) and *B. cinerea* (isolate 61-34) in water or Czapek-Dox broth (50%) plus yeast extract (0.05%) as related to time of storage of conidia in air prior to incubation. **4)** The effect of dilution of Czapek-Dox broth plus yeast extract (0.10%) on germ tube elongation of isolates 61-34 and 71-2 of *Botrytis cinerea*.

filter was placed over the top of the first filter. A buret containing either sterile glass-distilled water or sterile Czapek-Dox broth (50%) plus yeast extract (0.05%) was placed over the filter apparatus and adjusted to deliver 85 drops/minute (6.7 ml/minute). The liquid was drawn through the filters by suction at a rate which prevented accumulation of excess liquid over the filters. Conidia were incubated simultaneously in incubation dishes as controls. Following leaching, conidia (leached with water) were resuspended in sterile glass-distilled water which was pipetted into incubation dishes. Conidia that were leached with nutrients adhered to the filter as they germinated. They were prepared for observation by staining with cotton blue in lactophenol and then dried.

RESULTS

Germination (%) of conidia of all isolates of *B. squamosa* was equivalent in water and in nutrients, but germ tube elongation during the first 12 hours differed between isolates (Table 1). Germ tube length in water ranged from 40 to 123% of that in nutrients. The four isolates inoculated onto plants in water suspension all produced significant numbers of lesions, but produced more lesions per conidium with nutrients. Percent germination reached a maximum between 6 and 8 hours after the start of incubation (Fig. 2). The rate of germ tube elongation was greater in water than in nutrient solution during the first 12 hours of incubation (Fig. 1).

Percent germination of conidia of different isolates of *B. cinerea* was considerably less in water than in nutrients (Table 2). Germ tubes formed after 2 to 3 hours of incubation and ceased elongation in water after 4 hours. In nutrient solution, germ tubes elongated rapidly throughout the 12-hour period (Fig. 1). Most germ tubes produced in water were shorter than the conidia (Table 2). None of the isolates produced significant numbers of lesions on onion leaves inoculated with conidia in water, but all isolates produced expanding lesions when inoculated in nutrients (Table 2). The germ tubes produced in nutrients were thicker and stained more intensely with cotton blue than those produced in water. Conidia of both species swelled during 1-3 hours of incubation in either water or nutrients.

Incubation of mixtures of conidia of *B. cinerea* and *B. squamosa* in water or nutrient solution had no effect on germination (%) or germ tube elongation of either species.

Germination (%) of *B. squamosa* conidia was equivalent in water and nutrients regardless of age (Fig. 3). Germination (%) of 1-day-old conidia of *B. cinerea* was equivalent in water and nutrients, but more of the conidia that were 2 or more days old germinated in nutrients than in water (Fig. 3). Maximum germination (%) was approximately 60-70% for both species and declined with increased age, even in nutrient solution.

Germination (%) of *B. cinerea* conidia in vitro was unaffected by 10^{-3} dilution of the standard nutrient solution. Germ tube elongation was equivalent in water to that in the 10^{-4} and 10^{-5} dilutions (Fig. 4). Germ tube elongation was maximal in the 10^{-1} dilution. Lesion formation was increased only with undiluted nutrient solution and dilution of 10^{-1} and 10^{-2}.

Nearly 100% of conidia of *B. cinerea* germinated in the four nutrient sources, but germ tube elongation differed (Table 3). Germ tube elongation was greatest in cattail pollen filtrate and progressively less in standard nutrient

TABLE 2. Effect of exogenous nutrients[a] on germination (in vitro) of conidia and formation of lesions on onion leaves by *Botrytis cinerea* isolates[b]

Isolate	Germination (%)		Adjusted germination[c] (%)		Germ tube length (μm)		Lesion count[d]	
	Water	Nutrient solution	Water	Nutrient solution	Water	Nutrient solution	Water	Nutrient solution
71-2	44	99	8	99	7	87	0	183
70-8	51	99	12	99	9	72	0	97
70-11	55	99	11	99	7	79	...	...
70-25	55	97	12	97	8	71	...	...
70-37	55	99	19	99	12	91	0	133
71-5	60	97	12	97	8	67	0	100
71-4	64	99	21	99	12	80	...	...
72-13	65	97	26	97	12	95	...	...
70-29	66	97	19	97	10	71	...	...
71-1	69	99	24	99	10	78	...	...
72-7	69	100	17	100	9	78	...	...
72-17	72	99	14	99	9	81	...	...
72-19	72	98	37	98	16	81	...	...
72-11	75	99	42	99	14	76	1	142
72-2	77	99	37	99	13	90	0	148
70-35	78	100	29	100	12	84	0	242
61-34	79	99	39	99	15	90	0	363
70-33	82	99	28	99	12	81	...	...

[a]Czapek-Dox broth (50%) plus yeast extract (0.05%).

[b]Numbers presented are the averages for two independent experiments. For all isolates, differences between values in water and in nutrients were significant ($P = 0.01$).

[c]Percent of conidia with germ tubes longer than the conidium.

[d]Number of lesions per leaf. Differences between isolates were not significantly different ($P = 0.05$).

solution, lacunar diffusate, 1% glucose, and distilled water. The effect of the nutrient sources on lesion formation by *B. cinerea* was similar to the effect on germ tube elongation (Table 3). Although *B. squamosa* produced expanding lesions on onion leaves when sprayed in water, the addition of standard nutrient solution increased the numbers of lesions. Frozen cattail pollen filtrate significantly increased numbers of lesions. Addition of 0.5% glucose or lacunar diffusate diminished numbers of lesions (Table 3).

Conidia of *B. cinerea* produced on different media did not differ in germination in vitro or in lesion formation. Germination (%) of *B. squamosa* conidia produced on different media did not differ. Conidia produced on PDA had a significantly lower rate of germ tube elongation when germinated in water and produced fewer lesions when inoculated in water. However, when inoculated in nutrient solution, similar numbers of lesions were formed as from conidia produced on other media.

Conidia of both species did not germinate during leaching (6 hours) with glass-distilled water, but germinated normally when subsequently incubated in water or nutrient solutions (Table 4). However, both species germinated during the 6-hour leaching period with nutrient solution. Conidia of *B. cinerea,* which were leached with water and then placed in fresh water for 6 hours, produced short germ tubes equal in length to those produced by conidia incubated in water for 12 hours (Table 4). Both were significantly longer than those produced by conidia incubated in water for 6 hours. Many of the conidia of *B. squamosa* which failed to germinate following leaching appeared to have ruptured during leaching.

DISCUSSION

Botrytis squamosa and *B. cinerea* differ markedly in dependency on nutrients for conidial germination in vitro and lesion formation on onion leaves. Unlike *B. cinerea, B. squamosa* is capable of forming expanding lesions without exogenous nutrients, but the number of lesions was increased by addition of nutrients. *Botrytis cinerea* appears to be partially dependent on nutrients in vitro, but *B. squamosa* is not. The range of *B. cinerea* isolates tested in this study represents a spectrum of nutrient dependency. Blakeman (4) stated that different isolates of *B. cinerea* may be nutrient-independent or -dependent with respect to exogenous nutrient requirements for

TABLE 3. The effect of different media on germination (in vitro) of conidia of *Botrytis cinerea* and on lesion formation on onion leaves by conidia of *B. squamosa* and *B. cinerea*

	B. cinerea[a]		Lesions per leaf	
Medium	Germination	Germ tube length	*B. cinerea*	*B. squamosa*
Glass-distilled water	89	17	0 a[d]	9 a
Glucose solution[b]	100	39	5 ab	1 a
Lacunar diffusate	100	87	18 b	3 a
Standard nutrient solution[c]	100	100	85 c	24 a
Cattail pollen filtrate	100	130	84 c	128 b

[a]Values are expressed as percent of value in standard nutrient solution after 12 hours for three independent experiments because all treatments were not included in each experiment. Average values in standard nutrient solution were: percent germination = 100%, germ tube length = 99 μm.

[b]Glucose (1% w:v) was used for in vitro germination and 0.5% glucose (w:v) for inoculations.

[c]Czapek-Dox broth (50%) plus yeast extract (0.05%).

[d]Numbers in the same column followed by the same letter were not significantly different ($P = 0.01$).

TABLE 4. The effect of leaching with water or nutrient solution[a] on in vitro germination of conidia of *Botrytis cinerea* or *B. squamosa*[b]

	B. cinerea		*B. squamosa*	
Treatment[c]	Germination (%)	Germ tube length (μm)	Germination (%)	Germ tube length (μm)
H_2O, 6 hours	73 b	6 a	100 e	53 d
N[b], 6 hours	98 d	19 bc	100 e	38 c
Leached, H_2O, 6 hours	1 a	...	13 a	8 a
Leached, N[b], 6 hours	98 d	22 c	75 d	27 b
H_2O, 12 hours	90 c	17 bc	100 e	58 c
N[b], 12 hours	100 e	99 e	100 e	186 g
Leached, H_2O, 6 hours + H_2O, 6 hours	90 c	16 b	62 b	39 c
Leached, H_2O, 6 hours + N[b], 6 hours	99 de	31 d	66 c	77 f

[a]Czapek-Dox broth (50%) plus yeast extract (0.05%).

[b]Numbers in the same column followed by the same letter are not significantly different ($P = 0.01$).

[c]Conidia were incubated either in a leaching apparatus for 6 hours (leached), in incubation dishes for 6 or 12 hours (unspecified), or first in the leaching apparatus for 6 hours and then the incubation dish for 6 hours.

conidial germination. In this investigation, such distinctions between isolates of *B. cinerea* do not seem appropriate for two reasons: (i) germ tube elongation in water ceases soon after germ tube emergence even for isolates with high germination percentages; and (ii) when conidial germination by *B. cinerea* is compared to that for *B. squamosa,* differences between isolates of *B. cinerea* (percent germination and germ tube elongation in water) are insignificant compared to the differences between species. All isolates of *B. cinerea* appear to be at least partially dependent on nutrients by the latter criterion.

The difference in the nutrient dependency of the two species may relate to several factors. (i) The two species may differ in the quantity and/or quality of endogenous reserves of nutrients. The calculated volume of *B. squamosa* conidia (1,860 μm^3) is appreciably greater than that for *B. cinerea* (660 μm^3). Sensitivity to fungistasis has been found to be correlated inversely with conidial size (7). However, there was no correlation between conidial size and the amount of glucose required to promote germination (29). (ii) *B. cinerea* is known to produce an endogenous inhibitor of germination (8). The effects of a similar inhibitor in *Colletotrichum cingulata* were counteracted by nutrient addition (21). (iii) On the leaf surface, inhibition by leaf-surface waxes (4) or leaf-surface bacteria (3) may be counteracted by nutrients. (iv) Nutrients contained within the spores of some plant pathogens may be absorbed by the leaf (12, 15, 19). (v) Differences in growth habit of *B. squamosa* and *B. cinerea* on the onion leaf surface may contribute to differences in their pathogenicity (11).

The leaf also can be a source of nutrients for spores (2, 28). *Botrytis cinerea* requires a carbohydrate source for pathogenesis, but the effect of the carbohydrate in some instances is enhanced by other chemicals (1, 9, 20, 22). Amino-acid requirements, specifically for glutamine, may limit development of *B. cinerea* on beet leaves (6).

Onion leaf leachates have been reported to contain 7-20 μg carbohydrate per milliliter and 0.01-0.05 μmole of amino acids per milliliter (16). Yoder and Whalen (33) found that *B. cinerea* conidia failed to respond to glucose concentrations below 99 μg/ml and that for 100% germination 99,000 μg/ml was required. In the present study, 30 μg/ml of sucrose was required for any increase in germ tube length (maximal at 3,000 μg/ml). Maximum germination required 30 μg/ml and lesion formation required 300 μg/ml. These sucrose levels, however, do not take into account the probable effects of the other components of the standard nutrient solution. The nutrient status of the leaf surface in the presence of pathogens probably is not static, but may be marginally deficient in carbohydrate. Exogenously applied pectolytic enzymes may increase the permeability of onion leaves (11). Nongerminated conidia of *B. cinerea* contain endopolygalacturonase (32) which, if sufficiently active, could stimulate increased nutrient leakage and increased pectolytic enzyme production. Stimulation of *B. cinerea* by lacunar diffusates may explain in part why *B. cinerea* is able to destroy the leaf when injected into the lacuna even though it did not produce expanding lesions when sprayed on the leaf surface.

With increasing age, conidia of *B. cinerea* become more nutrient-dependent for germination (24). Procedures for harvesting and storing conidia in this study may have been excessively harsh as indicated by the low vigor of the conidia. The conidia of *B. squamosa* remained nutrient-independent despite their age and a general decline in vigor.

Unlike certain other fungi (13, 25), the substrate for conidia production had no effect on percent germination. *Botrytis squamosa* conidia produced on PDA may fail to accumulate endogenous nutrients or may take up an inhibitory substance, causing reduced germ tube elongation. The constitution of the substrate does affect the nature of materials taken into the conidia of *B. cinerea* (14). The substrates tested, however, were limited to those which supported production of conidia by *B. squamosa.*

The capability of conidia of both species of *Botrytis* to germinate after leaching with water indicates that endogenous reserves of nutrients were not depleted. However, both species were inhibited by leaching with water but not nutrients, suggesting that even the nutrient-independent spores are subject to fungistasis (5, 30).

Since glucose previously has been shown to stimulate *B. cinerea* (10, 20), the failure of glucose to stimulate lesion formation by *B. cinerea* and the inhibition by glucose of lesion formation by *B. squamosa* was unexpected, although similar findings have been reported (4, 23, 26). Factors which may have contributed to these effects are: (i) glucose may have selectively enhanced antagonistic saprophytes; (ii) glucose may have led to a nutrient imbalance; (iii) glucose may have provoked a host response deleterious to the pathogens; (iv) the amount of glucose may have been insufficient to stimulate pathogenesis; or (v) glucose may have repressed pectinase production.

The effect of environmental and cultural factors on quantitative and qualitative composition of foliar leachates may be of importance for the pathogenesis of both *B. squamosa* and *B. cinerea*. Bright light previously has been associated with increased amounts of carbohydrate in leachates (31) and increased lesion formation by *B. squamosa*. Differences in soil fertility also may affect leaf leachate composition and susceptibility to *Botrytis* spp. (27). Other factors which might enhance the availability of nutrients on the leaf surface include: herbicide and physical injury, guttation, length of wetting periods, alternation of wetting and drying periods, temperature extremes, water stress, and the activities of other pathogens.

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