The Role of Phenols in Botrytis Brown Stain of Onion

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ABSTRACT

Crushed, pigmented yellow onion scales inhibited germination of conidia of *Botrytis cinerea*. The amount of inhibition was correlated with the degree of resistance to brown stain of the onion cultivar employed. Conidial germination of *Colletotrichum circinans* was totally inhibited by crushed scales of all three colored cultivars, but not inhibited by the white cultivar.

Although catechol inhibited growth of both *B. cinerea* and *C. circinans*, the latter was inhibited at a much lower concentration. Colony development of *B. cinerea* was inhibited at a lower concentration than mycelial growth. Mycelial growth of *B. cinerea* was not inhibited by protocatechuic acid or quercetin.

When *B. cinerea* was grown for several days on agar containing catechol, a brown color developed in the medium identical to that on diseased onion scales. The color did not develop in media containing quercetin or protocatechuic acid nor when *C. circinans* was grown on catechol agar. Catechol probably functions as the substrate for the staining reaction. Furthermore, the fact that white onion scales do not inhibit *B. cinerea* yet do not become stained may be attributed to a lack of the substrate, catechol.

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Botrytis cinerea Pers. causes superficial flecking of onion *(Allium cepa* L.) leaves, colonizes senescing and dead leaves, and causes Botrytis brown stain on onion bulbs (2, 6). Brown stain normally appears as a superficial, dark-brown discoloration of the pigmented scales of the bulb, most frequently occurring on the neck and shoulder. In field trials (3), it was found that the yellow onion cultivars Elba Globe and Buccaneer were susceptible and moderately resistant (respectively) to brown stain, the red cultivar Southport Red Globe was more resistant and very little brown stain was found on the white cultivar Southport White Globe. This suggested the possibility that pigmentation may be related to susceptibility and resistance, as is the case for the onion smudge pathogen, *Colletotrichum circinans* (Berk.) Vogl. (12). Pigmentation in onion scales is due to the presence of quercetin (3,3′,4′,5,7-pentahydroxy flavone) and anthocyanins with which are associated the simpler phenolics protocatechuic acid (3,4-dihydroxybenzene carboxylic acid) and catechol (1,2-benzenediol) (1, 7, 8, 12). Taliyeva (10) found that anthocyanins from onion bulbs stimulated germination of conidia of *B. cinerea* while a breakdown product, anthocyanidan, was highly toxic. Catechol and protocatechuic acid are believed to be responsible for resistance of colored onions to onion smudge (7, 8). Phenolic compounds are involved in many brown discolorations of plants (5).

This study was undertaken to investigate the effect of dry, colored onion scales and the phenolics associated with pigmentation, catechol and protocatechuic acid, on growth and conidial germination of the brown stain pathogen, *B. cinerea,* and to investigate the role, if any, of catechol and protocatechuic acid in the reaction leading to stain development in the disease.

MATERIALS AND METHODS.—Sporulating cultures of *B. cinerea* isolate 61-34 were maintained by frequently transferring single conidia to potato-dextrose agar (PDA) slants which were incubated at 21 C with a 12-hour photoperiod of fluorescent light (Sylvania Cool-White FTOT12-CW) at a distance of 10 cm. Standard conidial suspensions were prepared by gently washing the surfaces of 2- to 8-week-old cultures with 10 ml of sterile distilled water. Mass transfers of conidia of *C. circinans* were made to PDA slants, and subsequent handling was as for *B. cinerea*.

The basal medium used for testing the toxicity of phenolic compounds consisted of agar, 20 g; dextrose, 10 g; peptone, 5 g; KH_2PO_4, 1.0 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g and 1,000 ml distilled water. Stock solutions of test compounds were added to the media at 52 C after

autoclaving to give desired final concentrations. Approximately 15 ml of each test medium was added to each of 10 sterile, plastic petri dishes (15 × 100 mm) per treatment.

Suspensions containing 20 to 50 conidia per ml were prepared by dilution of standard conidial suspensions in which the number of conidia was estimated using a hemacytometer. One milliliter of the diluted suspension was spread on the solidified agar in each of five dishes per treatment. The dishes were incubated in darkness at 21 C, and the number of resulting colonies counted.

Plugs of *B. cinerea* mycelium plus agar were cut with a no. 3 cork borer from the margins of three-day-old petri plate cultures and transferred to the center of plates containing the test medium. The plates were incubated in darkness at 21 C, after which measurements of the diameter of mycelial growth were made at two locations on the colony.

The effect of different colored scales on conidial germination was studied by the crushed-scale technique. Petri dishes containing one piece of bent glass tubing and a microscope slide were autoclaved and 0.1 ml of standard conidial suspension of *B. cinerea* was placed on each slide. Pieces of dry, outer scale and fleshy inner scale about 5×5 mm were removed from bulbs of the cultivars Southport Red Globe, Southport White Globe, Buccaneer, and Elba Globe, and crushed in separate drops of conidial suspension with a sterile scalpel. The plates were placed at 21 C in darkness for 18 hours, after which percent germination of conidia was determined by counting 100 conidia per replicate. Three replicates were used per treatment, and comparable measurements of the germination of *C. circinans* were made.

RESULTS.—Crushed inner fleshy scales of onion were totally inhibitory to conidial germination of both *C. circinans* and *B. cinerea* during the first 18-24 hours. Dry scales of all three pigmented cultivars inhibited conidial germination of the smudge pathogen, while those of the white cultivar did not (Fig. 1). Conidia of *B. cinerea* germinated equally well in the presence of dry scales of both the white cultivar and the brown stain susceptible cultivar Elba Globe, but percent conidial germination was slightly reduced in the presence of the moderately resistant yellow cultivar Buccaneer, and still further reduced in the presence of the red cultivar.

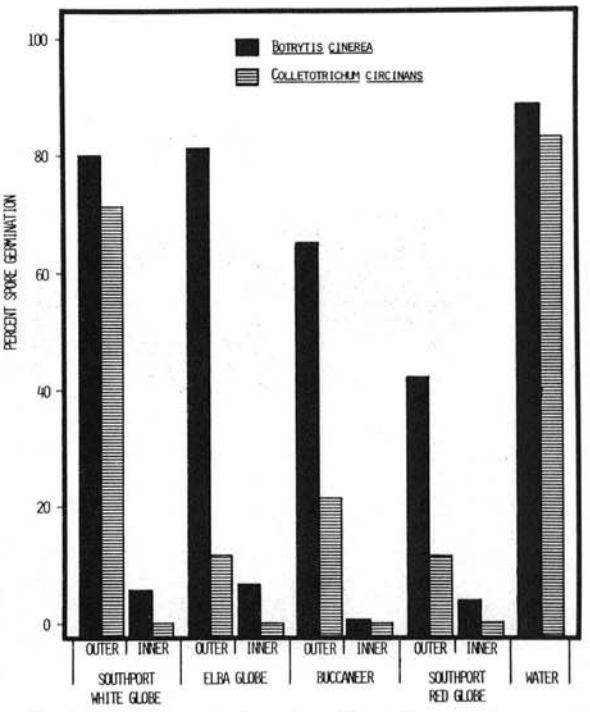

Fig. 1. Percent germination of conidia of *Botrytis cinerea* and *Colletotrichum circinans* in water drops containing crushed dry (outer) or fleshy (inner) scales of four different onion cultivars.

Table 1 shows the effect of catechol concentration on colony development and mycelial growth of both pathogens on agar. There was no effect on the number of colonies of *C. circinans* at 50-100 μg/ml; the number of colonies was reduced at 200-250 μg/ml; and no colonies developed at 300 μg/ml. The mycelial growth steadily decreased as catechol increased, with no growth occurring at 300 μg/ml. The number of colonies of *B. cinerea* was unaffected from 50-300 μg/ml of catechol, decreasing from 350-450 μg/ml and no colonies developed at 500 μg/ml. Mycelial growth of *B. cinerea* declined steadily as catechol was increased, with growth recorded at all concentrations tested.

TABLE 1. The effect of catechol concentration on number of colonies and diameter of mycelial growth of *Colletotrichum circinans* and *Botrytis cinerea* after 4 days on agar medium[a]

Catechol concentration (μg/ml)	Colonies per plate		Mycelial growth (mm)	
	C. circinans	*B. cinerea*	*C. circinans*	*B. cinerea*
0	61.8[b]	39.2	13.5	46.0
50	55.8	41.2	11.6	37.0
100	58.0	38.4	6.2	30.3
200	48.2	38.0	2.2	20.5
250	9.4	33.4	0.7	18.8
300	0.0	38.8	0.0	17.3
350	. . .	27.2	. . .	12.7
400	. . .	12.2	. . .	12.1
450	. . .	1.4	. . .	11.1
500	. . .	0.0	. . .	8.6

[a]Basal medium formulation: agar, 20 g; dextrose, 10 g; peptone, 5 g; KH_2PO_4 1.0 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; and 1,000 ml distilled water.

[b]Average of five replicates per treatment.

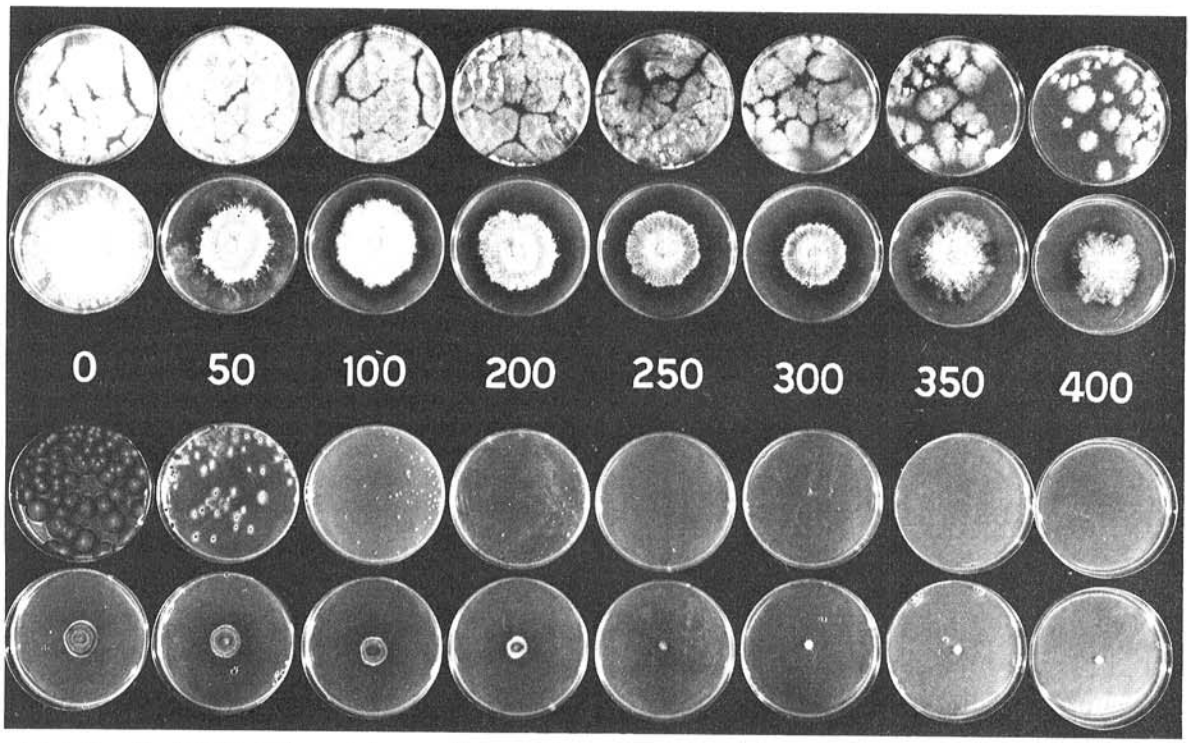

Fig. 2. Growth of *Colletotrichum circinans* and *Botrytis cinerea* on agar containing 0 to 400 μg/ml catechol. Rows from top to bottom; colonies of *B. cinerea*, mycelial growth of *B. cinerea*, colonies of *C. circinans*, and mycelial growth of *C. circinans*.

Colonies of *C. circinans* grew at a constant rate, and had negligible effect on the color of the media containing catechol from 0 to 500 μg/ml. The mycelial growth pattern of *B. cinerea* was dependent on the concentration of catechol. At concentrations to 300 μg/ml the mycelia displayed a constant growth rate. After 2 to 4 days a zone of deep brown-black color developed in the media. The intensity of the color appeared to increase in plates with progressively higher catechol concentration. Following coloration, mycelial sectoring occurred, particularly in plates with greater than 300 μg/ml of catechol. After coloration and sectoring, the growth rate at higher concentrations of catechol (350 μg/ml and higher) appeared to increase relative to the initial growth rate at the same concentrations (Fig. 2).

Protocatechuic acid up to 600 μg/ml did not inhibit mycelial growth of *B. cinerea*. With increasing concentration, colony diameter increased from 38.0 mm without protocatechuic acid to a peak of 44.7 mm at 300 μg/ml. A light-brown color developed in the protocatechuic acid medium after 2-4 days of incubation in the presence of the pathogen. When quercetin was added up to concentrations in excess of its solubility in water, there was no effect on mycelial growth of *B. cinerea* after three days of incubation. After five days of incubation, mycelial growth also was increased slightly above that on basal agar medium. The color of the quercetin medium was unaffected.

DISCUSSION.—Protocatechuic acid and catechol, both of which are associated with pigmentation in onion scales, were less inhibitory to growth on agar of *B. cinerea* than to *C. circinans*. In vivo, conidial germination by *C. circinans* was equally inhibited by scales of all three pigmented cultivars tested, while that of *B. cinerea* was only slightly inhibited by one of the yellow cultivars, and more significantly inhibited by the red cultivar. These data are in agreement with those of Walker and Lindegren (11) as regards *C. circinans*. However, they found total inhibition of conidial germination of *B. cinerea* in the presence of both red and yellow scales (11). This disparity may be due to the source and nature of the *B. cinerea* isolates used; their isolate was obtained from cyclamen, and it was nonpathogenic on onion. Our isolate 61-34 was isolated from onion, and it was capable of inducing brown stain on onion scales. It is possible that sensitivity to catechol may be a limiting factor in the virulence or avirulence of an isolate on onion scales. That spores of neither pathogen germinated in the presence of fleshy scales of any color, could be attributed to many factors. For example, since all four varieties of onion used were pungent, it is possible that the toxic allyl sulfides associated with pungent onions described by Owen et al. (9) inhibited germination.

Of the three phenolic compounds commonly associated with colored onion scales, it would appear that only catechol had a significant inhibitory effect in vitro. Quercetin and protocatechuic acid did not inhibit mycelial growth at similar concentrations.

The brown color produced when *B. cinerea* is grown on agar containing catechol suggested that the pathogen may cause the brown stain reaction by action on catechol in vivo. Preliminary work indicated that preparations from diseased tissue could catalyze the oxidation of catechol, but further work is needed in detailing the identity and

nature of this enzyme system.

Data are not available on the concentration of catechol, which occurs naturally in onion scales. Thus, it cannot be stated conclusively that this compound is responsible for resistance to brown stain in onion. However, since catechol is not known to occur in white onion scales, and because these scales did not inhibit conidial germination, the known presence of catechol in colored scales may account for inhibition of conidial germination by colored scales (8). White cultivars may not develop symptoms of brown stain because they lack catechol, an apparent substrate for the staining reaction. The quantitative determination of phenolics in onions of varying degrees of resistance, although not within the scope of this work, would appear to be a highly feasible and important area of future research.

That symptoms of Botrytis brown stain are so frequently associated with the neck of the onion bulb may be related to two factors; (i) the usual avenue of ingress for the pathogen may be through the leaf, with subsequent growth down into the bulb scale and, (ii) the concentration of phenolics is lower in the neck than the rest of the onion bulb (3, 12). The possibility that the dark band of staining, characteristic of this disease, may occur at the location where the pathogen first encounters high concentrations of phenolics (i.e., the shoulder of the bulb) should be investigated. It should also be determined whether or not the pathogen continues to grow within the scale after the occurrence of the dark band of staining.

A problem for further investigation is the possibility that polyphenols produced in the course of the disease might inhibit pectolytic enzymes of the pathogen as reported by Deverall (4) for the chocolate spot disease of broad bean caused by *Botrytis fabae*. A mechanism of this sort could explain why onion scale tissue appears intact despite the discoloration, and why lesion size is somewhat limited.

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