Symptomatology, Etiology, and Histopathology of Botrytis Brown Stain of Onion

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ABSTRACT

Botrytis cinerea was isolated with consistency from dry outer scales of onion bulbs displaying brown stain symptoms. Proof that *B. cinerea* was the causal organism of the disease was obtained by inoculation of healthy onion bulbs, brown stain symptom development, and subsequent reisolation of the fungus from the areas with symptoms. Several methods of inoculation and the symptoms induced are described. Spots produced on artificially inoculated dry scales were limited in size and remained physically intact. Fleshy scales were actively macerated prior to a general discoloration of the entire scale.

In naturally infected dry scales, the pathogen grew intercellularly with little visible damage to tissue structure. Hyphae were far more abundant in the mesophyll parenchyma than in other tissues of the bulb scale. Sclerotia were produced in the abaxial epidermis.

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In recent years in New York, a surface blemish of onion bulbs has been observed sporadically. The disorder produces a dark brown discoloration of the dry outer scales of onion bulbs. When severe, rejection of bulbs during commercial grading may occur. The literature on onion diseases does not reveal any previously known similar diseases. This study was conducted to determine the cause of the disease.

MATERIALS AND METHODS.—A 10% solution of commercial bleach containing 5% sodium hypochlorite was used for all surface sterilizations.

Isolation of the pathogen.—Disks were removed from dry outer scales of yellow and red onion bulbs with symptoms of brown stain using a No. 4 cork borer. The disks were wetted for 5 sec in 95% ethanol and surface-sterilized for 1 min. The disks were dried and two disks were plated on potato-dextrose agar (PDA) in petri dishes. The plates were incubated at 20-23 C, 50 cm below two fluorescent and two ultraviolet lights (Sylvania Lifeline F40-CW and Sylvania Lifeline F40-BLB, respectively) for 3 days. When conidiation occurred, single conidia were transferred to PDA slants. When only mycelial growth occurred, transfers were made from the margin of growth to PDA slants. All experimental cultures were routinely incubated on PDA slants at 20-22 C (12-hr photoperiod) 10 cm from one fluorescent light (Sylvania Cool-White F20T12-CW).

Botrytis cinerea Pers. was the only organism isolated consistently from onion scales with symptoms of brown stain. Isolation frequency was 60% in attempts made from both yellow and red scales. The fungus was identified following the description of Hennebert (3). All isolates obtained sporulated on PDA when grown as previously described.

Inoculations.—Different methods of inoculation were attempted. One set of bulbs was surface sterilized for 30 min and placed on metal racks in sterile casserole dishes containing 100 ml sterile distilled water. These bulbs were inoculated with *B. cinerea* (61-34), *B. allii* Munn (61-2), and *B. squamosa* Walker (64a), by first removing a square of tissue from the dry pigmented scales and the outermost fleshy scale and then inserting a square of the appropriate fungus growing on PDA where the scale had been removed. A square of sterile PDA was placed in the control bulbs and the dishes were placed on a laboratory bench (20-23 C) for 2 weeks.

A culture of *B. cinerea* (isolate 61-34), isolated

from onion, was used for all remaining inoculations and was maintained as described above. Monoconidial transfers were made 2-8 weeks before the culture was used. Conidial suspensions were prepared by adding 10-ml sterile distilled water and gently scraping the surface of sporulating cultures with a sterile transfer loop.

Entire bulbs, surface-sterilized and placed in casserole dishes, were inoculated using different forms of inoculum of *B. cinerea*. Green leaves of the onion cultivar 'Elba Globe' were excised and 6- to 8-cm sections surface-sterilized for 5 min. The leaf sections were then placed on moistened filter paper in a sterile petri dish and 1 ml of spore suspension was applied to the surface of each leaf segment. Sterile distilled water was applied to the controls and the leaves were incubated under the standard culture incubation conditions for 48 hr. Carborundum (400-mesh) was sprinkled on the surface of half of the bulbs, which were then abraded by vigorously rubbing the Carborundum into the outer scale. Equal numbers of wounded and nonwounded bulbs then were inoculated with either a drop of conidial suspension retained with a ring of petroleum jelly or with a 5- × 5-mm square of infected leaf tissue. The bulbs were incubated for 14 days at 20-22 C in a humid atmosphere.

In another experiment, nonwounded bulbs were inoculated using infected leaves as inoculum. All dry scales were removed; (i) at the time of inoculation, (ii) one week prior to inoculation, and (iii) two weeks prior to inoculation. Unpeeled bulbs also were inoculated.

Half-bulbs also were inoculated after the dry outer scales and the basal plate were removed and the bulbs sliced longitudinally in half.

The half-bulbs were surface-sterilized for 30 min, placed in casserole dishes, and 2 ml of spore suspension were applied to the cut surfaces. The half-bulbs were incubated for 14 days.

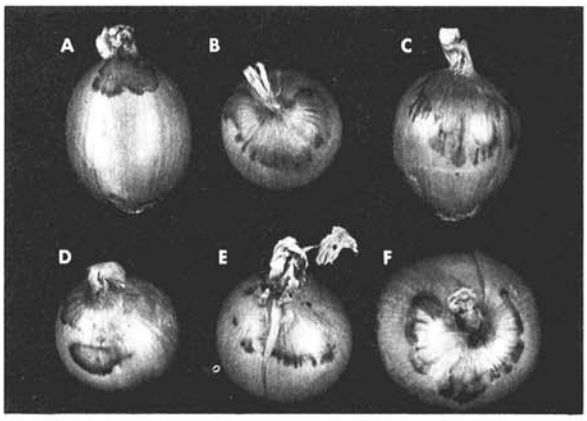

Fig. 1. Symptoms of Botrytis brown stain on the necks and shoulders of yellow onion bulbs. **A, B, E, F)** Typical neck symptoms with intense staining at the margin of the stain. **C)** Band of intense staining at a slight distance from the margin. **D)** A large spot stain on the median portion of the bulb with an intensely stained margin.

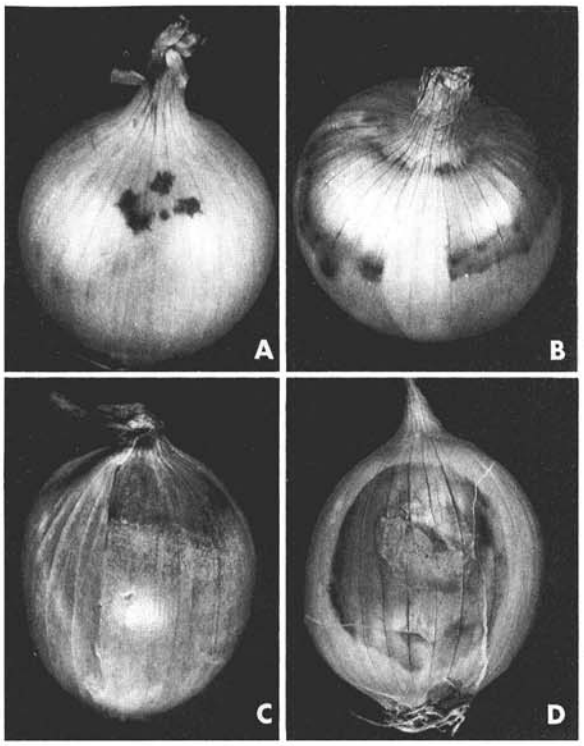

Fig. 2. A) Spots of Botrytis brown stain on a yellow onion. **B)** A portion of the infected dry scale was removed to reveal the immediately adjacent fleshy scale which was not affected by the pathogen. **C)** The right half of the outer dry scale was removed to reveal the second dry scale with extensive brown stain. **D)** Botrytis brown stain on a yellow onion inoculated at the center of the stain with *Botrytis cinerea.*

Histopathology.—The methods used for histological studies were adapted from Sass (4). Naturally infected dry scales from bulbs of the cultivar Elba Globe were fixed in formalin-acetic acid, dehydrated in a tertiary butyl alcohol series, embedded in paraffin and sectioned. Sections on microscope slides were stained in aqueous acid fuchsin and aqueous toluidine blue and mounted permanently.

RESULTS.—*Symptomatology.*—Brown stain on onion bulbs grown in western New York involved two distinct symptoms. 1) The most predominant was discoloration extending from the neck onto the shoulder of the bulb. The margin of the stain was dark brown to black. The central portion was a lighter brown (Fig. 1-A, B, D, E, F). In isolated instances the darkest portion of the stain occurred in a band slightly removed from the margin of the stain (Fig. 1-C). 2) Frequently dark brown to black circular spots 5- to 15-mm in diam were produced on the dry scales of the bulb. Such spots were not associated with particular portions of the bulb and were of a solid uniform color (Fig. 2-A). A few bulbs had larger spots with light brown centers and dark brown margins (Fig. 1-D).

TABLE 1. Incidence and severity of brown stain on onion bulbs inoculated by different methods with *Botrytis cinerea*

Condition of bulbs[a]	Inoculum[b]	Incidence[c]	Severity[d]
Wounded	Sterile leaves	0/18	0.00
Nonwounded	Sterile leaves	0/18	0.00
Wounded	Sterile water	0/18	0.00
Nonwounded	Sterile water	0/18	0.00
Wounded	Conidial suspension	5/18	0.39
Nonwounded	Conidial suspension	4/18	0.22
Wounded	Infected leaves	11/18	0.67
Nonwounded	Infected leaves	12/18	0.78

[a] Bulbs were wounded by abrading with Carborundum.

[b] Inoculum consisted of either segments of leaves infected by *B. cinerea* or suspensions of conidia from cultures in water. Sterile leaves or sterile water were applied to the controls.

[c] Incidence is reported as the ratio of number of infected bulbs to the number of bulbs inoculated.

[d] Severity is reported as the total severity rating divided by the number of bulbs inoculated. Severity ratings were determined by the following system: 0 = no brown stain, 1 = stained area 1-3 mm in diameter, and 2 = stained area greater than 3 mm in diameter.

Both spot and neck symptoms of brown stain occurred on red onion bulbs. The symptoms were similar to those on yellow onions with the brown discoloration distinguishable from the natural color of dry, dark red scales.

Brown stain symptoms on white onions occurred very infrequently. Both neck and spot symptoms occurred, but scales were stained an almost imperceptible light tan.

Generally, brown stain was restricted to the dry "wrapper" scales of the onion bulb. The internal, fleshy scales are apparently unaffected under normal growing conditions (Fig. 2-B). The pathogen appears incapable of penetrating laterally from scale to scale. Scales with brown stain are often either surrounded by healthy scales or enclose healthy scales (Fig. 2-C).

Sporulation of *B. cinerea* was infrequently associated with brown stain symptoms. Such sporulation was sparse compared to normal growth habits of the organism on other suscepts or in pure culture. Sclerotia were observed in the center of several spot stains. These were on the outer surface of the scales and were 1- to 3-mm in diam and less than 1-mm thick.

Inoculations.—Each of the three *Botrytis* species used to inoculate bulbs caused brown stain of the dry scales and dry rotting of the first fleshy scale. The only visible difference in pathogenesis of the three species involved the intensity of staining. *B. cinerea* caused the most intense discoloration (Fig. 2-D) followed by *B. allii* and *B. squamosa*, respectively.

Approximately two-thirds of the bulbs, on which leaves infected with *B. cinerea* were placed, developed symptoms; whereas, only one-third of those inoculated with spore suspensions developed symptoms (Table 1). Wounding had no discernible effect on disease development.

All the fleshy scales of inoculated half-bulbs were actively macerated by *B. cinerea*. Following a 7- to 14-day lag period after inoculation, the macerated scales began to discolor and within 2-3 days the entire half-bulbs were stained a dark brown.

Peeled bulbs were much more readily invaded by the pathogen than were intact bulbs. The time interval between peeling the bulbs and inoculation had no effect on the lesion size. Bulbs peeled 2 weeks prior to inoculation, generally appeared more intensely discolored (Fig. 3-D) than those peeled at inoculation (Fig. 3-B) or 1 week prior to inoculation (Fig. 3-C). Unpeeled, inoculated bulbs developed spots about one tenth the size of those on peeled bulbs. Spots on the former were much more intensely stained (Fig. 3-A).

Histopathology.—Sections of onion scales with brown stain reacted more intensely with toluidine blue than did healthy scales and showed extensive colonization by hyphae (average diam 5.8 μm) (Fig. 4-A). The hyphal diameter is similar to that reported for *B. cinerea* by Hennebert (3). No evident degradation of infected tissues occurred, but some intercellular spaces within the mesophyll appeared

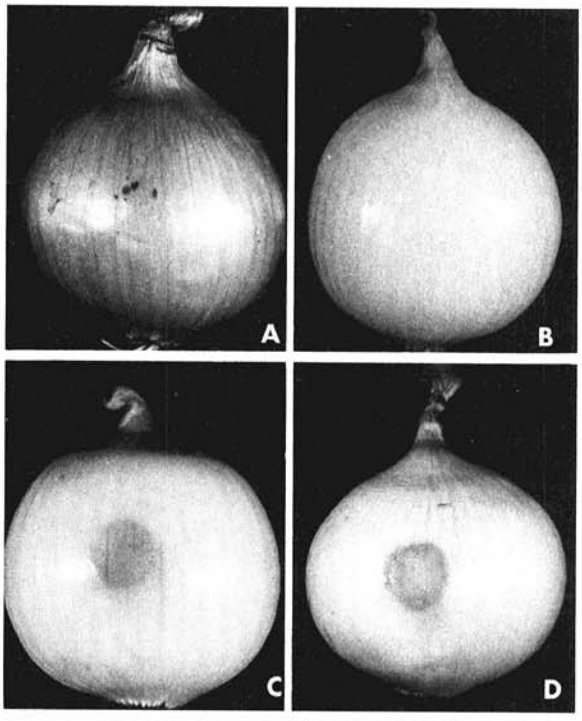

Fig. 3. Yellow onion bulbs inoculated with leaves infected with *Botrytis cinerea*. **A)** Intact bulb with several small spots of Botrytis brown stain. **B)** All dry scales were removed immediately preceding inoculation. The dry rotting of the fleshy scale is not readily apparent due to the lack of discoloration. **C)** All dry scales were removed 1 week prior to inoculation. A slight brown discoloration developed in association with dry rotting of the fleshy scale. **D)** Dry scales removed 2 weeks prior to inoculation. Color development was slightly more intense.

forcibly enlarged by the hyphae. Hyphae were found in the abaxial epidermis, the adaxial epidermis, the mesophyll, and the vascular tissues,but were of much greater predominance in the mesophyll parenchyma (Fig. 4-B). Hyphal growth occurred intercellularly (Fig. 4-C). Sclerotia were initiated within the abaxial epidermis (Fig. 4-D) and were produced on the outer surface of the scales. Hyphae were not observed to

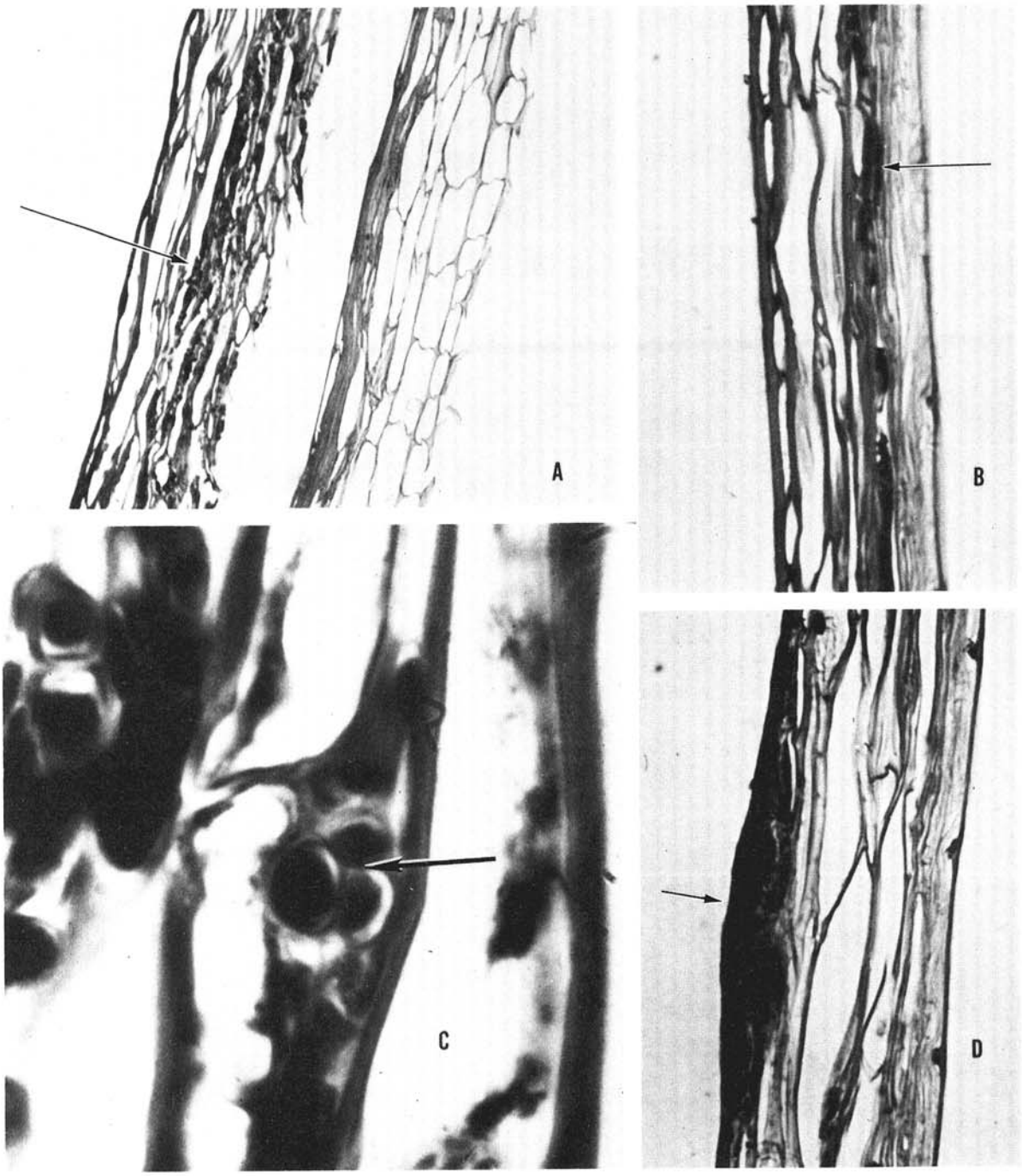

Fig. 4. A) Adjacent dry scales from a single onion bulb; healthy on the right and infected on the left with extensive network of ramifying hyphae (arrow) evident (Cross-section, × 92.5). **B)** Cross section of an infected scale showing predominance of ramifying hyphae (arrow) in the mesophyll tissue (× 92.5). **C)** Cross section of an infected scale showing hyphal ramification of an intercellular space (× 398). **D)** Cross section of an infected scale showing presence of a sclerotium (arrow) in the outer epidermis (× 92.5).

penetrate from one scale to an adjoining scale. The reason for this is unknown.

DISCUSSION.—*Botrytis cinerea* was shown to cause a new disease of onion for which the name Botrytis brown stain is suggested. Two other species of *Botrytis* (*B. allii* and *B. squamosa*) which cause bulb decay and leaf blighting of onion (5) also were capable of causing brown stain in artificial inoculations but were not isolated from naturally infected scales. This suggests that under field conditions *B. cinerea* is the natural pathogen.

It is suggested that the two distinct symptom patterns resulted from two distinct means of inoculation. The pathogen either directly penetrates the scale to initiate spot development or grows down into the scale from either the leaf sheath or the leaf proper to cause the neck-shoulder symptoms. *B. cinerea* has been shown to actively colonize senescent leaves (2). From such an infection, the fungus could further penetrate to the bulb scales. Such a mechanism could explain the occurrence of infected scales covered by unstained scales. If the fungus does not penetrate the scale directly, it might never come in contact with the external scale.

The results of inoculations of half-bulbs, whole bulbs peeled at different times prior to inoculation, and unpeeled whole bulbs suggest two physiological aspects of this disease. The first suggests that the staining reaction is dependent on the accumulation of a substance, possibly catechol, the substrate for the reaction itself. This would account for the lag period prior to staining of half-bulbs and the greater staining of bulbs peeled two weeks prior to inoculation (1). The second aspect relates to the variability in staining observed between the outermost fleshy scales of peeled onions which might have been due to varying accumulations of catechol resulting from different senescence stages at the time of inoculation. Once scales have senesced totally they apparently are not susceptible to colonization by *B. cinerea*.

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