Development of Apple Fruit Rot and Basidiocarp Formation by Schizophyllum commune

A. J. Latham

Assistant Professor, Department of Botany and Plant Pathology, Auburn University, Agricultural Experiment Station, Auburn, Alabama 36830.

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ABSTRACT

Basidiocarps of *Schizophyllum commune* developed on apples inoculated with mycelium or spores incubated approximately 70 days at 30 C in 1 to 7 ft-c of light. *S. commune* infected apples at sites of natural or artificial wounds. Golden Delicious and Red York apples rotted faster than other cultivars tested, and exhibited the most pronounced zonate symptoms. Phytopathology 60:596-598.

Rotting July Delicious apples that exhibited a surface symptom, zonate in pattern, were found in an apple packing shed in central Alabama during July 1967. The apples were incubated on moistened filter paper in culture dishes at laboratory temperatures of 22-33 C. After 42-56 days' incubation, a white, feltlike mycelial mat had formed over the apples; subsequently, the rotting apples were placed on a shelf where light intensity was low. After 90 days' incubation, basidiocarps of *Schizophyllum* were observed on the rotted fruit.

Bailey & Zeller (1) reported that *Schizophyllum commune* Fr. appeared during the autumn on green apples thinned from the trees early in the growing season. Cooke (2) reported that *S. commune* found on apples might be a saprophyte and not a primary parasite. Lovisolo & Lenzi (4) also observed *S. commune* basidiocarps on fallen apples and pears in orchards near Monferrato, Italy. They inoculated apples with mycelium of *S. commune* and presumed the resulting rot was due to this fungus, since mycelium from rotted tissue possessed clamp-cells characteristic of basidiomycetes. However, latent natural infections by *Penicillium* sp. and *Physalospora obtusa* also developed on the apples. Lovisolo & Lenzi (4) attributed the lack of typical fruiting structures on artificially inoculated apples to the difficulty of reproducing natural environmental conditions, and the use of mycelium instead of spores. The present studies were undertaken to define optimal environmental conditions for fruit rot and sporulation by *S. commune* on apples. A preliminary report has been made (3).

MATERIALS AND METHODS.—*S. commune* was cultured on V-8 juice (Campbell Soup Co.) agar to determine cardinal temperatures for growth. The agar plates were inoculated with 5-mm discs of *S. commune* cut from the periphery of rapidly growing cultures. Inoculated plates were incubated at 5-degree intervals from 0-45 C, with final determinations made by adjusting temperatures more closely near the cardinal growth points.

Preliminary inoculation tests either resulted in failure to produce basidiocarps or production of minute, stunted fruiting bodies on *Malus sylvestris* Mill. 'Richared', 'Red Delicious', and 'Golden Delicious' after approximately 70 days at 25 C. The sparsely produced, stunted fruiting bodies that developed suggested that environmental conditions were not conducive to development of normal basidiocarps on apples. Unfavorable light intensity was suspected, since cultural tests on V-8 juice agar had indicated similar problems. Cultures of *S. commune* on V-8 juice agar were incubated at 28 C in: absolute darkness; 20-200 ft-c (measured with a Weston Illumination Meter, Model 756, Quartz Filter) incandescent light, 8 hr/day; and in 20-100 ft-c for 5 min exposure per day (minimal incandescent light). Dark experiments were conducted in metal canisters placed in a blacked-out incubator and fungal development recorded after intervals of 12, 14, 16, 20, and 30 days using 6 cultures/sampling date. The influence of 20-200 ft-c light upon basidiocarp development was studied in temperature chambers equipped with 40w and incandescent bulbs. Variation in ft-c was caused by the angle of light striking the surface of the cultures.

The response of cultivars to infection by *S. commune* was studied with: Golden Delicious, Red Delicious, Jonathan, Rome, Red Steele, Winesap, and Red York apples 5.3 to 6.3 cm diam. Apples free of all visible blemishes were washed in soap and water and wiped thoroughly with a cloth saturated with 95% ethanol. A 7-mm mycelial-agar-disc cut from 7-day-old *S. commune* cultures was placed in a hole aseptically cut in each apple; subsequently, the apple plug was replaced over the inoculum and the surface sealed with Scotch tape. Each apple was incubated in a sterile 9.5 $cm^2 \times$ 7.5 cm plastic cup containing three moistened filter papers. The cups were placed in plastic bags and the ends fastened loosely. Four apples of each cultivar were incubated at each of the following temperatures: 21, 24, 27, 30, and 33 C.

A spore suspension containing 2.2×10^5 spores/ml from basidiocarps growing on V-8 juice agar was used in one test, and from apples in a second test. Twelve Golden Delicious and twelve Red York apples were positioned in cups, four with calyx end up, four with side up, and four with stem end up. Half the apples of each cultivar in each position were wounded 12 times on the topside with needle punctures; half were left unwounded. The apples were sprayed to runoff

with the spore suspension; the cups were then covered with the plastic bags and incubated at 30 C.

RESULTS.—*Growth and fructification on V-8 juice agar.*—Cardinal temperatures for growth of *S. commune* on V-8 juice agar were 5 and 43 C with an optimum at 33 C (Fig. 1).

A moundlike growth of fungal tissue devoid of basidiocarps grew over the inoculum disc in cultures incubated in the dark (Fig. 2-A). Basidiocarps were produced, however, on the inoculum disc under minimal lighting conditions, and numerous basidiocarp initials formed on mycelium in agar plates incubated at 20-200 ft-c with an 8-hr photoperiod. The latter were rudimentary and stipelike, and were randomly situated over the agar surface within 4 days. When these cultures were transferred and subsequently incubated at 20-100 ft-c for 5 min/day, the basidiocarp initials remained small or dwarfed (Fig. 2-B). Basidiocarps attained normal dimensions, however, when incubated at 20-100 ft-c for 5-min photoperiods/day (Fig. 2-C) from the start of the incubation period. Although occasionally discoid, the basidiocarps were similar in size and shape to those originally observed on the rotted apples in storage.

On V-8 juice agar under minimal lighting conditions, mature basidiocarps developed within 16 days at 24, 27, and 30 C. Twenty days and 35 days were required for them to form at 21 C and 18 C, respectively. After 35 days' incubation, two small basidiocarps were also observed at 15 C. At 33 C, one hymenial structure minus a stipe formed on mycelium that had grown over the agar surface and down through the area between the top plate overlap with the bottom plate. Basidiocarp formation did not occur at 36 C. After 35 days' incubation at 36 C, the cultures were moved to 30 C and incubated 60 days, but fructifications did not develop. Hymenia were usually palmate when formed in agar cultures under minimal light exposure, and occasionally discoid when light intensity was 100-200 ft-c.

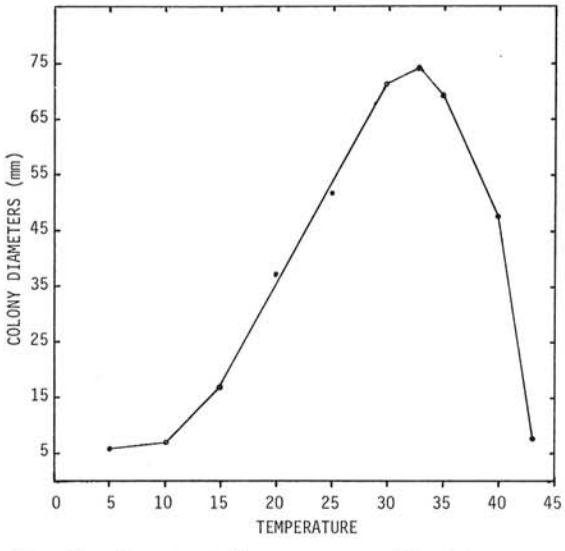

Fig. 1. Growth of *S. commune* on V-8 juice agar at various temperatures after 4 days' incubation.

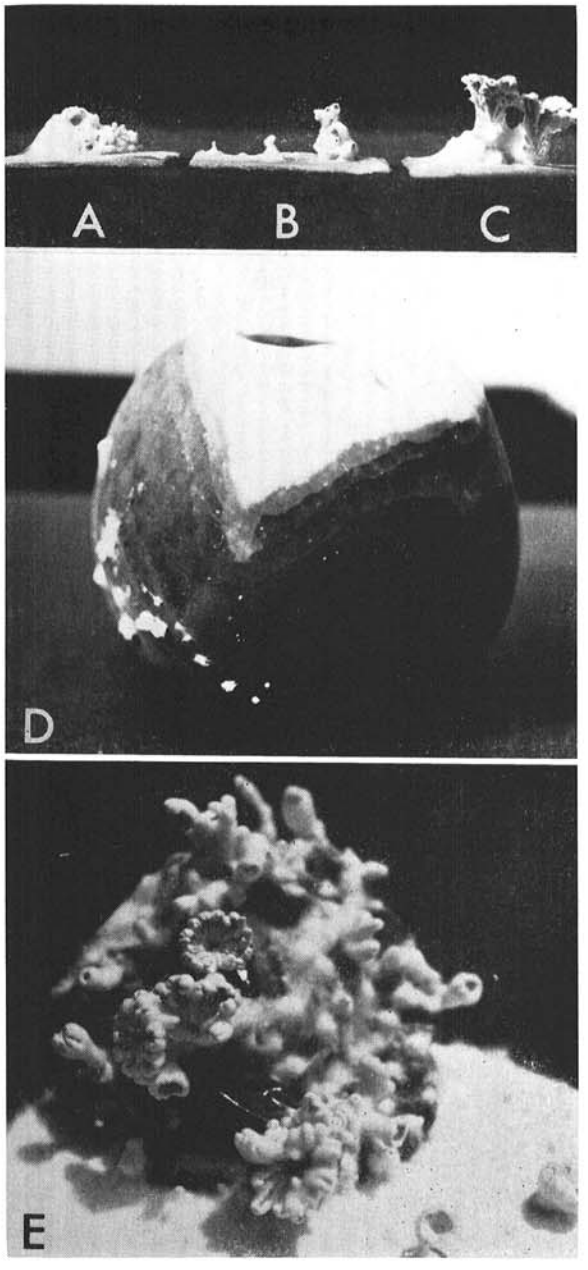

Fig. 2. A-C) Influence of light on *Schizophyllum commune* development; **A)** 30-day incubation with no light; **B)** 20-200 ft-c light 8 hr/day; **C)** 20-100 ft-c light 5 min/day. **D)** Symptoms of rot on Golden Delicious apple showing zonate pattern and mycelial tufts. **E)** Basidiocarps formed on inoculated Golden Delicious apple after 70 days' incubation.

Fructification on diseased apples.—Infected apple tissue became caramel-colored. Zonate or irregular bands (2-3 mm wide) formed in the surface of the fruit of Golden Delicious, Red Delicious, Jonathan, and Rome cultivars (Fig. 2-D); but not on Red

Steele, Winesap, or Red York at 30 and 33 C after 18 days' incubation. Golden Delicious and Red York apples rotted more rapidly than other cultivars, and were permeated by a spongy-textured rot 18 days after inoculation. After the apples were rotted, tufts of mycelium formed at irregular intervals on the surface. At temperatures optimal for rotting (30-33 C), mycelium enveloped the fruit within 56 days; at lower temperatures, apples became only partially covered by a mycelial mat.

On inoculated apples, fructifications did not develop at 33 C in two trials, and only two developed after 56 days' incubation at 30 C with a 5-min light exposure/48 hr. One apple of each cultivar was transferred after 56 days from each of the 30- and 33-C incubators to a laboratory shelf under 1 to 7 ft-c light/10 hr day at 25 C ± 5. Basidiocarp initials formed on Golden Delicious and Red York apples after 5 days; mature basidiocarps formed on all apple cultivars after 14 days. When the apples at 33 C were not transferred to 1 to 7 ft-c light (10 hr/day) until after 90 days' incubation, no basidiocarps formed; whereas, after 56 days' incubation, basidiocarps developed. About half the apples had basidiocarp initials after 90 days' incubation at 24, 27, and 30 C. These apples were then transferred to laboratory shelves under 1-7 ft-c of light for approximately 10 hr/day, and after 21 days (total incubation 105 days) an abundance of basidiocarps formed on all of the apples (Fig. 2-E). Apples from the 21-C incubators required 28-42 days' longer incubation to form basidiocarps.

Apples inoculated with spores produced on agar cultures or on apples developed infections at sites of wounds and natural openings. The optimum site of infection occurred at the calyx or stem end of the apples. Unwounded apples were infected only at the calyx or stem end. After 90 days' incubation, 92% of both Golden Delicious and Red York apples were rotted and covered with mycelial mats. Basidiocarps developed on the rotted apples after exposure to light. Small basidiocarps formed on apple petioles 21 days after the fruit were sprayed with spores. Palmate and discoid-shaped hymenia developed on apples and on filter paper saturated with leachate from rotting apples.

DISCUSSION.—Identification of *S. commune* from infected apples may be determined by isolation of the pathogen on V-8 juice agar, and incubation of the cultures at 28-30 C and light intensities of 20 to 100 ft-c for 5 min/day. Under these conditions, fructifications of *S. commune* develop on agar within 16 days. Light appeared to provide an initial growth stimulus for fructifications to develop; however, excessive light intensity caused an irreversible retardation of basidiocarp growth. Similarly, in the preliminary inoculations only rudimentary basidiocarps formed on mycelium-inoculated apples that were incubated under excessive light exposures.

The optimal time for basidiocarps to develop occurred after the apples were rotted and tufts of mycelium had developed on the apple surface or became enveloped by a mycelial mat. However, if sufficient light was not provided, the rotted apples passed through the fructification stage and did not produce basidiocarps.

The importance of this apple disease is unknown. *S. commune* is primarily a wood rotting fungus that commonly occurs on apple trees (2). Under conditions of proper humidity and basidiospore dispersal, infections may occur in wounds or in natural openings in the fruit such that the disease could become a problem.

LITERATURE CITED

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