Symptomatology of Orange Fruit Infected by the Citrus Greasy Spot Fungus

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ABSTRACT

A superficial blemish consisting of numerous minute black specks representing dead stomata is very common on orange rind in Florida. Oranges inoculated with the citrus greasy spot fungus, *Mycosphaerella* sp., developed similar symptoms. Although this fungus was frequently isolated from the rind of naturally affected fruit, it may not be the only local cause of stomatal necrosis. Phytopathology 60:1859-1860.

Greasy spot has long been known as an important disease of citrus leaves. The causal fungus was recently confirmed as a species of *Mycosphaerella,* but further taxonomic study will be required before the species involved in Florida can be designated (2).

Evidence is presented here that this fungus can also cause a blemish on orange rind by producing small black specks in the flavedo (Fig. 1-A). Individual specks are hardly discernible to the naked eye, but collectively they appear as an unsightly blemish, often further accentuated by delayed coloring of the rind due to retention of chlorophyll in adjacent living cells. The black specks consist of dead guard cells (Fig. 1-B), and frequently contain a few dead cells immediately beneath the substomatal chamber. Because stomata do not occur in those areas of the epidermis overlying oil glands, the black specks are restricted to portions of the rind between the oil glands.

Blackened stomata in the rind often contain fungal

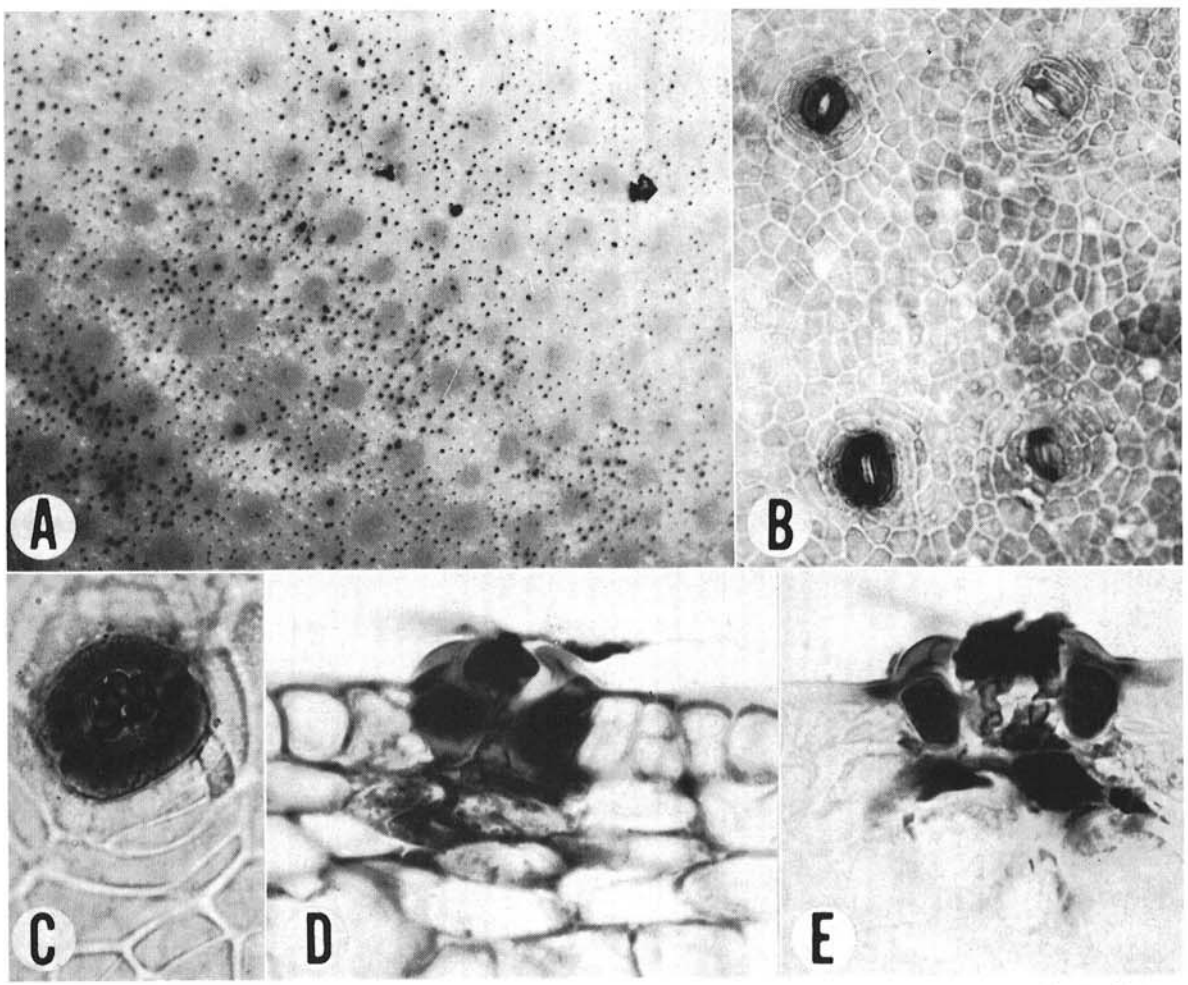

Fig. 1. Stomatal necrosis in orange rind. **A)** Numerous black specks restricted to areas between oil glands in a naturally affected Hamlin orange (×6). The larger lesions are due to citrus melanose. **B)** Portion of (A) magnified to show dead guard cells (×240). **C, D, E)** Injury to stomata and underlying cells following infection by *Mycosphaerella* sp. (×900). In (C), note dead guard cells and remains of fungus in outer stomatal chamber. In (D), note remains of extramatricular mycelium and fungal growth in outer stomatal and substomatal chambers. In (E), the section was cut obliquely through guard cells, and shows two hyphae growing through stomatal pore.

tissue in the outer stomatal chamber similar to that observed in leaves with greasy spot (J. O. Whiteside, *unpublished data*), with one or more hyphae growing through the stomatal pore; however, direct identification of the hyphae seen in the stomata as the greasy spot fungus was impossible, because this fungus produces conidia only from extramatricular hyphae and not from a stroma embedded in the host tissue (2). Therefore, isolations from the necrotic specks and inoculations with the greasy spot fungus were necessary to determine the etiology of these fruit symptoms.

Fungal isolation from orange rind.—Areas of rind containing numerous black specks were surface-sterilized by wiping with 95% ethanol and flaming. The rind was then placed under a stereoscopic microscope, and a flap of epidermis (including the guard cells) was lifted off the rind after making a tangential cut. A small portion of underlying tissue, about 0.1 mm^3, was then picked out with the point of a scalpel and transferred to cornmeal agar (CMA). These isolates were identified on the basis of conidial morphology, and identification was confirmed by pathogenicity tests on citrus leaves (2). The fungus was also isolated, although less frequently, from tissue beneath apparently healthy stomata.

Fruit inoculations.—Container-grown Pineapple orange trees were transferred from outdoors to the greenhouse in early April soon after fruit set. Fruit were inoculated in June with isolates of the *Mycosphaerella* obtained from infected orange rind and diseased leaves. Suspensions of fragmented mycelium and conidia were prepared from CMA cultures (2). A very thin layer of absorbent cotton 2 × 10 cm was then dipped in the inoculum and placed on the fruit. Noninoculated fruit were treated similarly, but with cotton soaked only in water. To prevent rapid drying, the fruit were enclosed individually in transparent polyethylene sleeves. These were tied to the stem behind the fruit and left open at the distal end, which extended about 10 cm beyond the fruit. A double layer of cheesecloth was wrapped around the plastic sleeve to prevent excessive heating. The cotton was kept moist by spraying with water every 2 days. The covers and cotton were removed after 14 days; and thereafter the fruit were kept dry.

Blackened stomata (Fig. 1-C) similar in appearance to those seen on naturally affected fruit (Figs. 1-A, B) were first observed in the rind in November when the fruit started to color, and appeared on fruit inoculated with either leaf or rind isolates. Out of 54 attempted reisolations from subepidermal tissue beneath black specks, 16 yielded the greasy spot fungus, as compared with three isolations of this fungus out of 54 attempts from beneath apparently healthy stomata of the same fruits. No symptoms developed on the noninoculated check fruit. The sections through the affected epidermis illustrated in Fig. 1-D, E were prepared after embedding rind in paraffin wax, sectioning at 10-μ thickness and staining with thionin for detection of dead cells, as described by Sadik & Minges (1), except that the period of immersion in the 0.05% thionin solution had to be reduced to 10 sec to avoid excessive staining of living cells.

Although it has been confirmed that the greasy spot fungus can cause minute black specks on orange rind, all necroses of the guard cells and underlying tissues are not necessarily the result of such infection. Nevertheless, fungal structures as seen in inoculated fruit have been observed in many samples of fruit showing stomatal necrosis; however, it was evident that stomatal penetration by this fungus did not always produce disease symptoms.

LITERATURE CITED

1. Sadik, S., & P. A. Minges. 1964. Thionin for selective staining of necrosis in plants. Amer. Soc. Hort. Sci. Proc. 84:661-664.
2. Whiteside, J. O. 1970. Etiology and epidemiology of citrus greasy spot. Phytopathology 60:1409-1414.