A Leaf Spot Disease of English Holly Caused by a Species of Sclerophoma

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A species of *Sclerophoma* was found to cause a leaf spot known as holly scab on English holly (*Ilex aquifolium* L.) in the North American Pacific Northwest. The holly scab spots are irregular, translucent to reddish-black, swollen areas found principally on the lower leaf surface, although some varieties show spotting on both surfaces (Fig. 1). The leaf spot is the product of hypertrophy and extreme cellular proliferation within the leaf mesophyll (3).

Extensive isolations were made from leaves from different holly cultivars collected in Oregon during the summer and fall of 1961. Affected leaves were washed in running tap water for ½ hr, then sections containing individual leaf spots were cut into 5-mm² segments. The segments were surface-sterilized in 5% Clorox (5.25% sodium hypochlorite) solution for 5 min and then further subdivided into smaller segments approximately 2 mm². These pieces were then placed in petri dishes containing either 2% potato-dextrose agar or water agar and held at room temp. Once fungus growth was observed, hyphal transfers were made to fresh plates.

Of the many different fungi obtained, none had ever been proven to be pathogenic on holly leaves; therefore, inoculation trials were initiated to test pathogenicity of the isolates. In all inoculation trials, comparable 2-year-old trees of the cultivar Brownell Special were used. Prior to inoculation, all trees were placed in a continuous-mist chamber at approximately 25 C. Small squares of agar and fungus mycelium 5 × 5 mm were removed from petri dish cultures and placed on the lower surface of the leaves of the test plants.

Inoculated trees were left in the moist chamber for an additional 24 hr, then removed to a greenhouse where they were held under observation. The agar with fungus inoculum was left on the leaf until it became desiccated and either fell off or was brushed off.

Of the 27 isolates tested in this manner, only one produced distinct holly scab symptoms after a 3-month period. The resultant leaf spot was typical of holly scab found under field conditions. Examination of leaf cross sections showed the same abnormalities described by Herridge (3) for field-infected materials. These included: (i) presence of hyphae in the abnormal tissue; (ii) considerable deposition of resinous materials and accompanying necrosis; (iii) extremely proliferated spongy mesophyll and resulting obliteration of intercellular spaces; (iv) degenerate or absent chloroplasts in infected tissues; and (v) an intact upper and lower epidermal layer.

Sporulation had not been observed under either field or laboratory conditions. The presence of extremely dimorphic mycelia in older cultures was noted and, as a consequence, the fungus was tentatively identified (1) as *Cuticularia ilicis* Document. This dimorphism is expressed by the occurrence of young mycelium that is generally hyaline, quite uniform in diam, and somewhat irregularly septate, while the older mycelial form is dark brown, very irregular in diam, and highly septate.

Subsequent exposure of cultures to near ultraviolet irradiation from Sylvania Blacklite-Blue, F40 BLB lamps (12 hr/day at 24-inch distance) stimulated the production of densely opaque pycnidia from older mycelium. These pycnidia vary in size from 75 to 200 μ and are usually oval but occasionally pyriform and lack an ostiole. The young pycnidia are very membraneous and of uniform density throughout. At maturity they become dense and dark. Pycnidia are usually formed separately rather than in clumps or aggregates.

Spores are single-celled, hyaline, ovoid to oblong, 5-7 × 2-4 μ and are immersed in a hyaline mucus that is readily dissolved in water. Many spores are distinctly guttulate, having one or two oil droplets.

Suitable living fungus material was sent to B. C. Sutton of the Commonwealth Mycological Institute in London. Dr. Sutton concluded that the holly scab organism was a *Sclerophoma*; therefore, he placed the isolate under accession number IMI 107649 and designated it as a *Sclerophoma* sp. While the author was visiting the Institute in 1967, the diagnosis was confirmed by G. Morgan-Jones, the specialist in the Sphaeropsidales at that time.

Available literature lists three species of *Sclerophoma* as having been described (2), none of which have been proven experimentally to be plant parasites or reported on *Ilex*. The holly pathogen most closely resembles *S. pithyophila* (Cda.) Hoehn., which has only been reported as a saprophyte on coniferous materials, except that pycnidial measurements do not compare closely. Leach (4) has demonstrated, however, that in some instances pycnidia formed under near ultraviolet irradiation are significantly smaller than pycnidia formed under normal conditions. Therefore, the variation in size of pycnidia would hardly seem an adequate basis to justify a species difference.

However, the holly scab fungus is the first demonstrated plant pathogenic *Sclerophoma*. On this basis and on the basis of morphological differences it may be a new species. It appears at this time, however, advisable to make careful morphological and pathogenic comparisons of the holly *Sclerophoma* with the known species of *Sclerophoma* before establishing a new species.

Holly scab has been demonstrated to be a fungus-induced condition, but the organism has not been found on holly sporulating under natural conditions, and the mechanism by which the fungus overwinters and is spread under natural conditions is not known. The possibility of ascigerous stage has been considered but not observed.

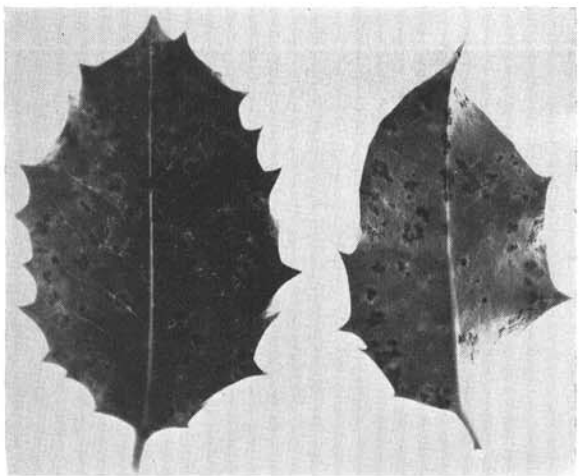

FIG. 1. *Sclerophoma* symptoms on *Ilex aquifolium* 'Brownell Special'. Left = upper leaf surface; right = lower leaf surface.

From inoculation trials, however, one can speculate on the nature of disease development. Under artificial conditions, distinct symptoms developed 3 months after inoculation. Healthy plants placed in the field in June showed holly scab symptoms by September. This indicates that holly scab inoculum is present in June.

The form of inoculum and manner of dissemination must at this time be a matter of speculation due to the absence of fruiting bodies, sexual or asexual, on field-infected leaves throughout the year. Because of the copious production of spores in pycnidia in culture, it is postulated that spores spread by wind or splashing rain water are the principal agents of infection.

Working from this assumption, prevention and control of the holly scab disease must then be centered around the time of inoculum dispersal and the emergence of new growth on the trees. Because both occur in the spring and early summer, a spring or summer spray program seems logical. Preliminary control tests were inconclusive.

LITERATURE CITED

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