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English holly culture in Oregon has been threatened by the development of red leaf spots which prevent sale of cut holly. Chemical, mechanical and insect injury and boron deficiency have been shown in the past to cause different types of red leaf spotting, but the cause of holly scab, a widely prevalent type of red leaf spotting, had not been determined. The objectives of this study were to determine the cause of holly scab and to investigate the factors that influence its development.

Holly scab spots are irregular, translucent, or red-black swollen areas principally on the lower leaf surface, although some varieties show spotting on both leaf surfaces. Observation of scab development on trees in a variety plot showed that the size and color of holly scab spots varies considerably on different varieties of holly. Green stem hollies appear to be as susceptible to scab as the brown or blue stem varieties but form less pigmentation. No

promising resistant varieties were observed.

Nutritional vigor of the host did not affect susceptibility to infection. When nitrogen, calcium or boron deficient plants and normal plants were exposed to natural inoculum in the field all plants became infected.

Over 100 different organisms were isolated from leaves affected with holly scab and inoculation attempts were made with 28 of the isolates. Only a species of Sclerophoma produced holly scab. Attempts to identify the fungus repeatedly lead to Phoma and Phyllosticta. Because of significant differences from characteristics of these genera, however, cultures were sent to B. C. Sutton of the Commonwealth Mycological Institute for identification. Sutton acknowledged that the fungus was not a Phoma or Phyllosticta. He pointed out its similarity to Sclerophoma pithyophila (Cda.) Hohn. and listed it as a Sclerophoma species with accession number IMI 107649.

The fungus in culture has pycnidia that are non-ostiolate, spherical to pyriform, ranging in size from 75 microns to 200 microns. Spores are hyaline, ovoid-oblong, guttulate, 2-4 x 5-7 microns with no visible sporophores at maturity but are embedded in a gelatinous matrix. The fungus grows slowly on streptomycin-PDA over a range of 15° to 25° C but does not grow at 30° C. It grew best on media with an initial pH from 4 to 7.

The holly Sclerophoma did not sporulate under normal laboratory conditions, but sporulated readily when KNO_3 was used as a nitrogen source or following exposure to prolonged periods of near-ultraviolet. Sporulation has not been observed in infected leaves.

Comparison of the holly Sclerophoma with descriptions of the Sclerophoma species listed by Grove showed distinct differences in host range and morphology which would support the establishment of a new species. Unfortunately cultures of other Sclerophoma species were not available for comparison, and further attempts to clarify the taxonomic status of the holly Sclerophoma are pending receipt of collections from Europe.

Attempts to control the disease, with spring and summer sprays were inconclusive due to a lack of natural infection within the test plots, but provided information on three other non-pathogenic leaf-spots. Purple blotch is a physiological imbalance that shows only with the ripening of the berries and colder weather. Copper injury occurred when fixed copper was used at normal recommendations in spring and fall spray applications. Leaf spotting associated with summer application of nabam was observed and is being studied further.

A LEAF SPOT DISEASE OF ENGLISH HOLLY CAUSED BY AN UNDESCRIBED SPECIES OF *SCLEROPHOMA*

by

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A LEAF-SPOT DISEASE OF ENGLISH HOLLY CAUSED BY AN UNDESCRIBED SPECIES OF SCLEROPHOMA

INTRODUCTION

English holly (Ilex aquifolium Tourn L.) is one of many kinds of specialty crops that have contributed to the diversity of ornamental horticulture in the Pacific Northwest. Recently holly has assumed a leading role in the Oregon greens industry. The last census in which it was recorded was in 1957; at that time the holly acreage in Oregon was listed at approximately 1300 acres.[1] This figure was for holly orchards only and did not take into account most holly nursery plantings. Undoubtedly, the acreage has increased, for it is not uncommon to see plantings of young trees in the north coastal areas of the state and in the Willamette Valley.

English holly is not native to this region but was introduced from England and Europe. One of the first recorded importations was as early as 1878 when Joseph Patrick Cronin had holly plants brought "around the horn" to be used in the landscaping of his home in Portland, Oregon (51. p. 3). Undoubtedly other similar importations occurred during that era. These, and importations by nurserymen, served as sources for the further propagation, selection, and

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[1] Conklin, Melvin J., Asst. Ag. Economist, O. S. U. Private communications.

establishment of English holly in the northwest, first as a landscape plant and later as a horticultural specialty crop.

The genus Ilex belongs to the family Aquifoliaceae. About 300 species are distributed principally throughout North and South America and temperate Asia and a few occur in Africa, Australia, and Europe (2, p. 629). Of these many species, only two are of economic value and cultivated to any extent in North America. These are the American holly (I. opaca Ait.), grown principally on the eastern coast, and English holly (I. aquifolium Tourn. L.), grown principally in the Pacific Northwest. The major difference between the two species is the occurrence on I. aquifolium of flowers in axillary clusters on one year old branches, while the flowers of I. opaca occur in one to few-flowered axillary solitary cymes on the current years growth (2, p. 628). Both species vary considerably in growth habit and have numerous varieties.

Many varieties of English holly are cultivated in Oregon and are used in the production of Christmas holly and/or as young ornamental planting stock. New varieties are continually selected for their different leaf, berry, and stem characteristics. Because the market demand is for a plant of exceptional aesthetic appeal, the occurrence of discoloration, wounds, or other unsightly blemishes detracts from the plant's value.

Several conditions in the Pacific Northwest detract from the commercial value of holly in this manner. One of the more serious problems to develop within the past ten years is the holly leaf spot condition, first described as scab by McWhorter in 1938 (27). These spots are superficially rough, raised and blister-like and may detract greatly from the desirability of holly as an ornamental material.

Recent research at Oregon State University has shown that similar types of spots may be caused by fungi, chemical injury, mechanical injury, boron deficiency, and insect injury. The holly scab condition has been attributed at various times to one or more of these causes. This investigation was initiated to determine and characterize the cause of holly scab.

REVIEW OF LITERATURE

The scab disease of holly was first reported and illustrated in 1938 by McWhorter (27). At that time, however, no effort was made to determine the cause of the rough, raised, blister-like spots which developed principally on the lower leaf surface. A photograph of this condition, taken by McWhorter in 1938, is shown in Figure 1a.

Several types of leaf disorder other than holly scab are now known to occur on English holly. A leaf and twig blight caused by a previously undescribed species of Phytophthora was reported in 1954 (6, 7). At the same time, reference was made to other types of leaf spots for which the cause was not known definitely. Research was directed during the next few years toward determining whether the various types of spots were caused by pathogenic organisms, insects, chemical injury, nutrition, or environmental factors.

Literature reports on holly leaf spots up to 1961 were discussed in some detail by Herridge (18). The most prevalent and misidentified is the leaf spotting caused by mechanical injury, where spines on one leaf puncture or scratch other leaves. Cork cells develop in response to the wounding and may form a red corky-like spot. Such spots normally can be identified by the puncture but may result from a very minor injury that cannot be detected by the naked eye (Figure 9c).

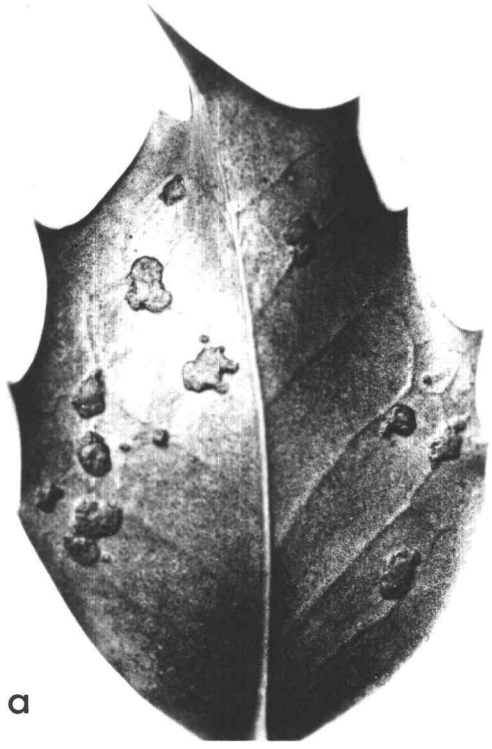
Phytophthora ilicis Budd. and Young causes a dark brown or black leaf spot that usually develops along the leaf margin at the base of a spine. Phytophthora leaf spot also results in severe defoliation and in many cases is a limiting factor in holly production.

Physiological factors also can result in leaf spotting. A red target-like spot on the under side of the leaf (Figure 1c), and a somewhat diffuse purple spot on the upper surface of leaves may develop when boron is deficient (Figure 1d). In some instances, the deficiency also manifests itself as a collapse of the mesophyll in younger leaves (Figure 1b). Symptoms of boron deficiency are distinctly different from the scab-like spot originally reported by McWhorter.

Chemical damage often results in severe spotting. Until recently, copper damage (Figure 9a) was frequently confused with the holly scab. In 1960, Herridge and Lambe (20) reported on spray trials conducted at Oregon State University where definite leaf-spotting was observed on English holly that had been sprayed with fixed-copper compounds in April and June. Copper injury causes the development of discrete, circular, slightly raised red spots on the lower leaf surface that, with experience, can be distinguished readily from scab (Figure 1a).

Another spot of holly leaves may be associated with midge injury (6, p. 14; 47, p. 71). A circular discolored area usually

Figure 1. Holly leaf spots. (a) Holly scab on field collected leaves. (b) Boron deficiency symptoms showing collapse of leaf mesophyll on young leaves. (c) Boron deficiency symptoms on lower leaf surface, showing target like spotting. (d) Boron deficiency symptoms on upper leaf surface, showing diffuse pigmentation. (x 1.5.)



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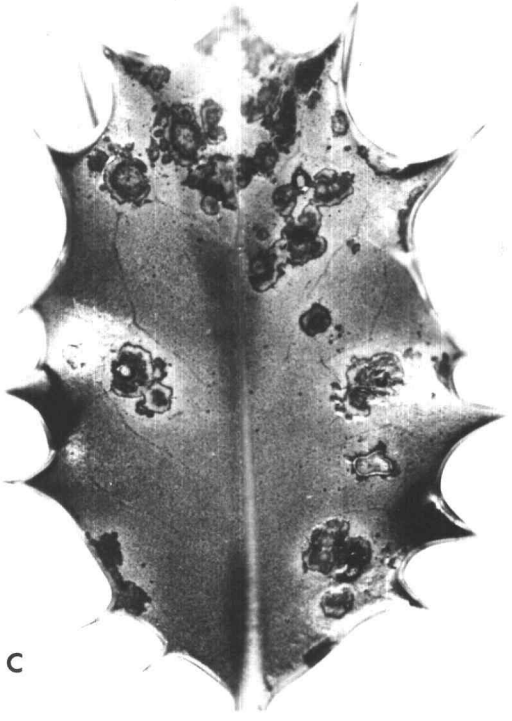

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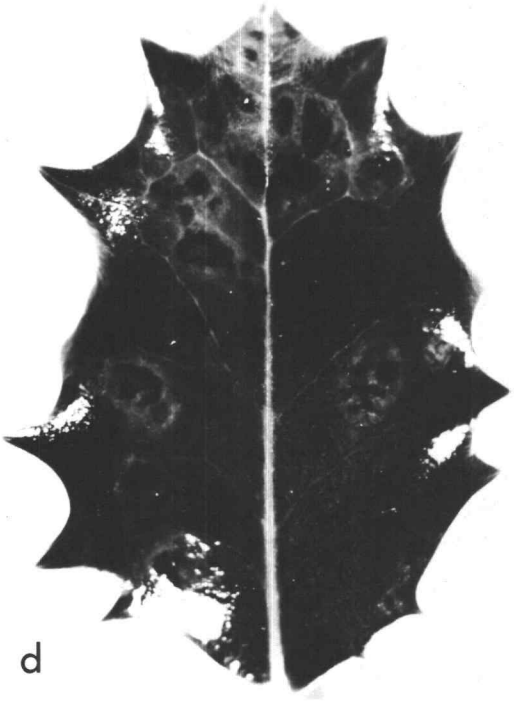

d

develops on the lower side of the leaf around the point of feeding injury. Frequently a dead midge is observed in the center of the spot. Welton (47, 48) also observed the seasonal activities of two species of eriophid mites in various locations throughout the state of Oregon. He found no relationship between the insects and the occurrence of holly scab.

McWhorter reported in 1938 (27) that he did not detect visible mycelial or fruiting bodies associated with the holly scab except for two instances in which a "Cladosporium-like" fungus was observed. In 1955, Buddenhagen (6) again pointed out the unsightly blemish and indicated that it appeared to be more prevalent in commercial plantings. These reports stimulated investigations of the cause of red leaf spots on holly by Anne E. Herridge. Herridge (18, 19) studied the normal and abnormal anatomy of the holly leaf with particular reference to the various types of leaf spots, among them the holly scab. She showed signs of fungus growth within malformed tissue in naturally affected leaf material collected from orchards, but was unable to reproduce these conditions with any of the organisms isolated.

Herridge found that the raised blister-like leaf spots were caused by an extreme proliferation of the spongy mesophyll cells. She pointed out that in the spring some leaves were observed to have very small spots visible only on the abaxial surface which, with the

use of a binocular dissecting microscope, appeared as dark spots in the regions of the stomata. She also maintained that fungal hyphae could be observed in the regions of the stomatal opening. She observed significant differences in leaf spot appearance, that varied with time of collection, location and variety.

In summarizing her observations of field collected materials, she (18, p. 28-29) stated that, "In general, fungal spots are always swollen and rough, at least on the abaxial surface, and may be single or aggregated. Chlorosis and translucency are early symptoms which are often followed by a slightly red discoloration before the intense red-black color appears in the center of the spot. The internal structure of these spots always includes irregular hypertrophy and proliferation, at least in the spongy mesophyll around the stomata, but may spread into the palisade and hypodermal layers. Chloroplasts are always absent in the enlarged cells and necrosis may be found according to the state of development. Cell wall thickening and warts may also be present. Stomatal penetration by fungal hyphae is not always apparent but intercellular mycelium of pectinaceous substances in adjacent host cell walls. In advanced stages, the hyphae may appear inside collapsed hypodermal and epidermal cells."

Herridge isolated several organisms and attempted limited inoculation trials with them. These organisms belonged to the

genera Phomopsis, Boydia, Gliocladium, Asteroma, Pullularia, Coniothyrium, Alternaria, Stemphylium, and Epicoccum, (18, p. 29). All these organisms failed to produce typical leaf spots.

Although only a few organisms have been found to be pathogenic on holly leaves and twigs, several have been isolated and described. Guba and Stevenson (17, 1. 21) found Phomopsis oxyspora Petrak on dead twigs of Ilex verticillata from Maryland and a Phomopsis sp. causing a die-back on Ilex sp. in New Jersey. McWhorter reported (26) that Phomopsis crustosa Trav., the imperfect state of Diaporthe eres Nits., was associated with a brown canker and die-back of branches of Ilex aquifolium in Oregon and Washington. This is the only report of this condition in the United States and no inoculations into healthy plants were attempted at that time. Grove also reported Phomopsis crustosa Trav. on dead or fallen leaves and twigs in England (15, p. 134). Other reports (43, p. 51; 17, p. 9, 12; and 49, p. 21) in the U. S. of this organism are obviously based upon the original report of McWhorter. Buddenhagen, in a series of inoculations using Phomopsis, was unable to obtain infection (5, p. 21). The relation of Phomopsis to scab development therefore is questionable. Although Phomopsis has been found in association with some types of spots, inoculation attempts have not been successful.

Boydia insculpta (Oud.) Grove, was reported (5, p. 6) as

destroying twigs of Ilex aquifolium in Great Britain and northern continental Europe. This organism was reported for the first time in Oregon in 1939 on Ilex aquifolium (28). Recent work has shown that this fungus is a secondary invader and only saprophytic upon the leaves of holly which are attacked by Phytophthora ilicis Budd. & Young (8). Boydia invades the tissue after it has been killed by P. ilicis and fruits abundantly in the diseased tissue. Boydia is easily identified by its long bi-cellular ascospores which are distinctly constricted at the septa. Other reports (43, p. 51; 17, p. 8; and 49, p. 21) of this fungus causing a disease are probably based on the original report.

Asteroma ilicis Grove is reported as causing irregular brown spots on living leaves of Ilex aquifolium in England (16, p. 145). Members of the genus Asteroma generally produce dark, small, globose, ostiolate pycnida, that are located in a mass of dark radiating hyphae and have one celled hyaline, ovoid to cylindrical conida (16, p. 166). Another report exists of an Asteroma ilicis by Roumequére (41, p. 218). According to Herridge both descriptions are nomena nuda so it is impossible to determine whether or not Grove and Roumequére are describing the same fungus (18, p. 32).

Coniothyrium ilicinum Ell. & F. W. Anderson has been reported on leaves of Ilex opaca from Maryland, New Jersey, and New York (17, p. 18). Herridge reports that no leaf spotting was obtained on

Ilex aquifolium with the Coniothyrium isolate that she used in inoculation studies (18, p. 38). The genus Coniothyrium Sacc. produces black, globose, separate, erumpent, ostiolate pycnida which have small, dark, one-celled, ovoid or ellipsoid conidia (3, p. 176).

Stagonsospora ilicis Grove is reported as occurring on leaves of English holly (16, p. 347) and has been intercepted on importations of Ilex from England (17, p. 22). According to Barnett (3, p. 184) the genus has dark, septate, superficial or erumpent, globose, ostiolate pycnidia with dark, two-celled conidia.

Grove reported several species of Phyllosticta (Pers.) Fr. as causing leaf spots of holly in England. P. aquifolina Grove (16, p. 23) shows spots that are at first small and round, then irregular, 1-3 mm. across, greyish-fuscous with a dark brown border. P. hynaldi R & S (16, p. 24) produces varying kinds of spots that range from small circular ones to ones that are larger, up to 10 mm. across, sinous in shape, at first purplish but later becoming cinerous with a fuscous-brown border. P. ilicicola Fr. (16, p. 24) is reported as causing spots on leaves of holly but apparently is confused with a couple of species of Phoma as well as being considered the imperfect state of Physalospora ilicis (Fr.) Sacc.

In the United States, seven different Phyllosticta species have been reported on various species of Ilex. P. concomitans Ell. & Ev. was reported on leaves of Ilex decidua from Louisiana (17, p. 21).

Driver (11) reported both P. haynaldi on leaves of Ilex cornuta var. burfordi Defr. (11) and P. ilicicola Ilex cremata (12) in Georgia. P. haynaldi has been reported also on leaves of Ilex verticillata in West Virginia (17, 22) and Ilex aquifolium in Denmark and France (17, p. 24). An undetermined Phyllosticta species has been reported on Ilex aquifolium in New Jersey, California, and Washington (17, p. 21).

Phyllosticta opacae Ell. & Ev. is reported to cause a leaf spot of Ilex opaca in the area of West Virginia to South Carolina to Texas and in California on an undetermined species of Ilex (17, p. 21).

Phyllosticta terminalis Ell. & G. Martin is reported as causing a leaf spot on I. cassina and I. opaca in the gulf states (17, p. 21). It should be pointed out that P. ilicicola (Cke. & Ell.) Ell. & Ev., P. prini Pk., and Phyllostictina ilicicola (Cke. & Ell.) Hoehn. are all imperfect or conidial forms of Physalospora ilicis (Fr.) Sacc. (17, p. 21) which is described as causing leaf spot, twig die-back, and branch cankers on Ilex aquifolium, I. coriacea, I. opaca, I. crenata, and I. verticillata in various areas of the east and southeast (17. p. 12). Driver (12) reports it as causing the death of stems and leaves with an accompanying defoliation on I. crenata var. rotundifolia in Georgia. In this report, the symptoms described do not appear to be similar to the blister leaf spot condition of the present study.

Phoma ilicicola (Desm.) Sacc. is apparently synonymous with the Phyllosticta ilicicola previously discussed and in turn is the imperfect form of Physalospora ilicis (Fr.) Sacc. Grove reports (16, p. 86) it on leaves and dead twigs of Ilex aquifolium. He also lists a Phoma ilicis (16, p. 87) and a Phoma santonensis (16, p. 88) on I. aquifolium in England.

In this country, there is a report (17, p. 20) of Phoma ilicis Desm. var. ilicis-opacae Sacc. on leaves of Ilex opaca in New Jersey. Phoma ilicina Ell. & F. W. Anderson, the perfect stage of Sphaeropsis ilicina (Ell. & F. W. Anderson) Kuntze, is listed by Guba and Stevenson as occurring on dead leaves and twigs of Ilex aquifolium and I. opaca in Maryland, New York, New Jersey, California and the Pacific Northwest (17, p. 20).

Phoma phacidiella (Cke. & Ell.) Sacc. is synonymous with Macrophoma phacidiella (Sacc.) Ber. & Vogl. and causes a leaf spot on I. opaca in New Jersey and Tennessee (17, p. 20), and I. aquifolium in Washington (44, p. 141).

To differentiate between various species of Phoma and Phyllosticta is rather difficult and much confusion may be found in the literature pertaining to these two genera. In general, with respect to host response, the Phyllosticta species usually cause necrotic spots and attack principally leaves, while the Phoma species are believed to be found principally on plant parts other than leaves and seldom

cause necrotic spots (3, p. 162).

Cercospora ilicicola Maubl. has been reported (17, p. 18) as causing leaf spot of Ilex opaca in South Carolina, Texas, and Georgia. This is considered to be a questionable report and the fungus is probably Cercospora ilicis-opacae Chupp which is reported from Georgia and South Carolina as causing circular white spots, 1-3 mm. in size and with purple borders, on the leaves of Ilex opaca (17, p. 18).

Cercospora ilicis Ellis is reported as causing small brown leaf spots on Ilex decidua, I. glabra, I. opaca and I. verticillata in the area from the gulf states to New Jersey (17, p. 18). Cercospora pulvinula Cke. & Ell. has been reported in that area as causing large, indistinct brownish blotches on the leaves of Ilex cassine and I. opaca in the same region (17, p. 18). This species is probably synonymous with the C. ilicis that is reported on I. verticillata.

Grove found Ceuthospora phacidioides Grove on dead fallen leaves of Ilex aquifolium (16, p. 289-290). Guba and Stevenson (17, p. 18) and Shaw (43, p. 51) reported this fungus as being in the Pacific Northwest and British Columbia, respectively. Grove described two kinds of stroma for this fungus, the first very small and Phoma-like, and the second larger, up to 1-1.5 mm in diameter. He maintained that the former is what has been sometimes called Phoma ilicis Ren. while the latter is the pycnidial stage of

Phacidium multivalve Fr. which has been questionably reported on Ilex opaca in America (17, p. 12). Guba and Stevenson list four other Phacidium species as present in America (17, p. 12). These are: Phacidium elegantissimum Berk. & Curt., on leaves of Ilex opaca in North Carolina; Phacidium sphaeroideum Cke. & Ell. on Ilex glabra in New Jersey; and a doubtful report of Phacidium ilicis Lib. on Ilex opaca. These three seem to be of little significance as compared to Phacidium curtisii (Berk. & Rav.) Luttrell which has been reported to cause a tar spot disease of Ilex opaca and I. decidua (17, p. 12). This fungus causes tiny yellow, chlorotic areas in the leaves at first; the symptoms then progress through a reddish-brown to a final glossy black spot. Stromata appear in the autumn with ultimate development of orange-red apothecial discs arising through the ruptured epidermis. The disease ultimately results in defoliation of affected leaves (25). Phacidium curtisii is synonymous with Macroderma curtisii (Berk. & Rav.) Hoehn., and Rhytisma curtisii Berk. & Rav. (17, p. 12).

Elsinoe ilicis Plakidas is reported to cause spot anthracnose of Chinese holly, Ilex cornuta, in the Gulf states (38). The organism attacks leaves, shoots and berries, apparently causing two kinds of injury. One, which is prevalent in the fall, is restricted to the upper leaf surfaces and appears as small black spots which may unite to form irregular black patches covering half the leaf surface. The

second type occurs on the upper surface in early summer and later shows on the lower leaf surface. These lesions are chlorotic at the beginning, later forming irregular brown patches. Perithecia, and the imperfect acervuli, are observed finally as compact stromatic bodies breaking through the cuticle. It is interesting to note that Plakidas' inoculations under conditions of high humidity did not produce lesions but rather many localized spots. This organism has as an imperfect stage, Sphaceloma, a fungus similar to Gloeosporium and Colletotrichum (3, p. 200).

As previously mentioned, the original report of scab on leaves of Ilex aquifolium in the Pacific Northwest was made by McWhorter in 1938 (27). He described the condition as rough, raised, scabby spots. In two of his collections from separate ends of the Willamette Valley, he observed a "Cladosporium-like" growth. This was confirmed by Anna E. Jenkins in personal communication with Dr. McWhorter. In examination of Dr. McWhorter's personal files and herbarium specimen #11028, it is quite obvious that the material he examined and that of the present work are identical. There are no other reports of Cladosporium on holly other than those host indices which base their listing on the original report. This Cladosporium-like fungus sporulating on field-collected materials was observed in the course of this study, but was not found to be pathogenic.

In 1963, Brown (5) tentatively identified an isolate obtained from English holly as Cuticularia ilicis Ducomet (9, p. 232; 42, vol. 22, p. 1502). A red blister-like leaf spot typical of scab was reproduced by inoculation. Cuticularia was reported by Ducomet (13) as the cause of a leaf spot on Ilex aquifolium in France as early as 1906.

Ducomet maintained that the mycelium remained in the epidermis and caused the round swollen spots by a mechanical expansion of the fungus itself. Ducomet also reported that the fungus digested its way through the cuticle into the epidermis. This histological description does not compare favorably with that of Herridge (19) who, as previously stated, described cellular proliferations as the cause of swelling.

Ducomet's description of the fungal hyphae is more significant. He observed a dimorphism of the hyphae in which the early stages were extremely simple but in older material became more complex, opaque, and irregular. It is now apparent that the chlamydospores of Brown's (5) description are the older, more complex and irregular mycelium of Ducomet. In no instance did Ducomet obtain either sexual or asexual sporulation. Brown reported no sporulating forms in his observation, other than the misidentified "chlamydospores".

The genus Sclerophoma von Hohnel should also be considered in this review. This genus resembles Phoma in many respects, but differs in the absence of a distinct ostiole. The pycnidia are very

thick-walled, the parenchymatous outer layer being dark brown and lined within by a well-developed "stromatic tissue" consisting of pale cells similar to those which generally form the main mass of a sclerotium. There are usually no sporophores, the spores being seated directly on the stroma which may at length resolve itself into a mucilage in which the spores are imbedded.

By this last character, Grove (16, p. 155) distinguished Sclerophoma from Plenodomus but he further stated that both were chiefly made up of species of Phoma which have assumed the sub-sclerotioid state through some influence of nature and evolution. The example that he used was Sclerophoma pithyophila (Cda.) Hoehn. which shows such an abnormal development that if the same fungus were observed in its normal state it would be called Phoma acicola.

The only Sclerophomas to be reported from the Pacific Northwest are Sclerophoma pini (Desm.) Hoehn, (16, p. 192) reported on various Pinus species, and Sclerophoma pithyophila (43, p. 192) which was reported as Dothichiza pithyophila (Cda.) Patr. on Libocedrus, Pinus, and Pseudotsuga (44, p. 55).

Chemical Control of Diseases of English Holly

Chemical spray programs have been developed for control of the Phytophthora leaf spot and twig blight and of green algae on English holly in Oregon (52). The Phytophthora disease and algae are

controlled by a fixed copper spray such as tri-basic copper sulfate at a rate of two pounds per hundred gallons of water or with a nabam and zinc sulfate schedule. Trees are sprayed when the cool, rainy weather begins in the fall, generally in mid-October or later. These spray programs have not been effective, however, in controlling holly scab.

The control recommended (25) for tar-spot on Ilex opaca caused by Phacidium curtisii is to avoid crowding of plants, remove lower limbs, destroy old leaves, and apply dichlone to the ground and plants at 10-14 day intervals in the spring. Tar-spot control with copper sprays in May and late June is reported in South Georgia (10). Also Bordeaux, Phygon XL, and ziram were applied both alone and in combination. The best results were obtained with Bordeaux alone and Bordeaux followed by Phygon XL in the later summer.

Leaf spotting caused by Phyllosticta species can be controlled by use of Bordeaux or any copper spray in the later summer and in early fall (30, p. 420). Driver (11, 12), who has done the most work with the various species of Phyllosticta on holly,, has not reported any attempts at control.

Simpson reported that, under Indiana conditions, zineb applied twice on the new growth in the spring controlled three distinct leaf spotting conditions on American Holly (45, 46). In correspondence

from Mr. Simpson[1], he stated that the holly scab is not one of them and further that he has never observed the condition.

Welton (47, p. 20) pointed out that trees which were sprayed with an application of diazinon and later with fixed copper sulfate in June of 1961 showed leaf spotting the following September that was smaller and much less prevalent than on unsprayed trees just 12 feet away. At that time he could not tell whether the diazinon or the copper was responsible. It should be pointed out, though, that spraying with diazinon alone never effectively reduced the holly scab condition.

Therefore, there is some indication that a copper spray applied in late May or early June may greatly reduce the severity of scab but the optimum time for application has not been worked out.

Copper Phytotoxicity on English Holly

Copper fungicides, while highly effective in controlling Phytophthora leaf spot and algae, may cause severe phytotoxic responses in holly. Under certain conditions, serious defoliation and leaf spotting have been reported by growers.

Herridge and Lambe reported (20) development of red spots on holly following application of tri-basic copper sulfate during April

[1] Simpson, Robert C., Simpson Orchard Company, Inc., Vincennes, Indiana. Personal correspondence dated March 24, 1965.

and June of 1958. The damage was directly proportional to the concentration of the spray material. The leaf spots were discrete, smooth, round, and seldom aggregated (Figure 9a). They were dark red on the lower surface and only slightly swollen. Occasional discoloration without swellings appeared on the upper surface and was usually associated with spotting on the lower surface. The internal disturbance was always in the region of the substomatal cavity where cork formation, hypertrophy and proliferation were observed. Necrosis occurred only when damage was far advanced. This type of damage was reported with both tri-basic copper sulfate and copper sulfate used at concentrations ranging from two to eight pounds.

There appear to be no other reports of chemical phytotoxicity on English holly. The work cited here was done in the spring and early summer while copper is normally used in the fall under somewhat cooler conditions. Therefore, adequate reports of copper phytotoxicity under normal usage are still lacking.

Oedema

Plakidas described a physiological condition on camellia leaves as being characterized by prominent corky outgrowths (34). The condition has also been observed on cultivated rhododendrons. (49, p. 133). The similarity of this condition to holly scab should not be overlooked.

At first, this condition was described (1, 31, 32) as being caused by a Sphaceloma species because, under conditions in Louisiana, this organism was consistently isolated from such spots. Plakidas described six distinct types of leaf spotting on camellias in 1948 (31) and Sphaceloma was consistently isolated from all of them.

When material obtained from California was used for isolations, Sphaceloma was not isolated even though symptoms in Louisiana and California were identical. Also, no inoculations using Sphaceloma were positive. This, along with histological examinations that showed no fungi in the affected tissue and the failure of spring spraying with Bordeaux to control the condition, lead Plakidas to re-examine the condition and describe it as physiological (36).

"Oedema", as this corky excrescence is termed, is believed to be associated with fluctuations in soil moisture and transpiration during mid-summer through early fall.

Good control of this condition has been obtained by using Dowax 222 emulsion in a foliar spray at three to four week intervals from early June through mid-September (36, 37). None of the trees sprayed with Dowax, one part in 25 parts of water, showed significant spotting. In contrast, the unsprayed trees and those trees sprayed with a copper-zinc-manganese fungicide became moderately to severely spotted with corky excrescences.

In material collected in the Portland area this past year a leaf spot of camellia and rhododendron has been identified[1] as the "oedema" of Plakidas' description. Comparison of this material with the holly scab condition indicates that the two forms of leaf spotting are not related.

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[1] Plakidas, A. G. Professor Emeritus Plant Pathology, Louisiana State University, Baton Rouge, Louisiana. Personal correspondence dated September 10, 1964.

GENERAL METHODS AND MATERIALS

This section contains a description of those experimental methods used commonly in the various sections. Modifications and specific methods are described in each section.

Media Preparation

Three media were used predominantly in these experiments.

Potato Dextrose Agar (PDA) was used most frequently for isolation and propagation of fungi. The two percent potato dextrose agar was composed of 20 grams of dextrose, 17 grams of agar, and the liquid broth of 200 grams of potato boiled in one liter of distilled water. This medium was autoclaved at 15 psi for 20 minutes, then divided into two batches. To one batch was added enough streptomycin to equal 100 ppm actual streptomycin per liter of medium. To the second batch, three drops of a 25 percent lactic acid solution were added to each 20 ml of median as it was poured into the petri dishes.

Fungi were maintained on strep-PDA in test tubes sealed to prevent mites from contaminating the cultures.

Water Agar, used in isolations and in obtaining single spore cultures, was prepared by adding 20 grams of agar to one liter of distilled water and autoclaving at 15 psi for 20 minutes.

Basal Semisynthetic Medium was used in all instances where specific nutrients were required. Also, it was used frequently as a liquid culture medium. The formulae for this medium and a micro-element solution were taken from Lilly and Barnett (24, p. 427).

Basal Semisynthetic Medium Components

Carbon source (generally dextrose)	10 g
Asparagine	2 g
K_2HPO_4	1 g
$MgSO_4 \cdot 7 H_2O$	0.5 g
Biotin	5 ug
Thiamine	100 ug
Micro element solution	2 ml
Distilled water to make	1 liter

Micro Element Solution

The following were dissolved in 600 ml distilled water:

$Fe(NO_3)_3 \cdot 9 H_2O$	723.6 mg
$ZnSO_4 \cdot 7 H_2O$	439.8 mg
$MnSO_4 \cdot 4 H_2O$	203.0 mg

C. P. sulfuric acid was added to make a clear solution and the volume was made up to 1 liter with distilled water.

When necessary, the pH of the medium was adjusted with the citrate phosphate buffer described by Gomori (15, p. 138). The

medium was then sterilized by running it through a millipore filter, type HA, with a pore size of 0.45 u, into pre-sterilized 125 ml Erlenmyer flasks. The filter was contained within a Millipore microsyringe holder xx30 025 00 attached to an Adams manual Aupette mounted on a ring stand. The mounting of the Aupette facilitated freedom of movement so that the cotton plugs could be removed, flask mouth flamed, media injected into the flask, and the plug replaced. The medium was then allowed to stand for a few days before inoculation to insure that no contamination had taken place.

Sectioning Procedures

Fresh materials were sectioned by a free-hand method in which the leaf sample was mounted in a split-carrot-block and cut with a razor blade. After sufficient practice, good sections were obtained. This method was frequently used when a rapid answer was needed in examining both inoculated and naturally affected leaves for fruiting stages of the fungus and the condition of the leaf. Sections were examined both in water and in lactophenol-aniline blue mounts.

Material for permanent mounts was sectioned in paraffin. Leaf sections containing a leaf spot were cut into approximately 10x10 mm squares and placed into a killing and fixing solution immediately. Frequently, this was done in the field at the time of collection. If not, the intact leaves were placed in plastic bags and returned to

the laboratory.

Herridge (18, 19) used both a formalin-acetic-alcohol and a Havashin's-acetic-formalin solution for killing and fixing holly leaves. She found the latter to be more successful so this mixture was used. The formulation was as follows:

Solution A		Solution B	
Chromic acid	4 g	Formalin	160 cc
Glacial acetic acid	40 cc	Distilled water	240 cc
Distilled water	360 cc		

Equal parts of the two solutions were mixed together at time of killing and fixing affected tissue. When possible, a vacuum pump was used to increase the rate of penetration of the killing and fixation solution. The tissue was left in this solution for 24 hours. After fixation, the material was washed thoroughly, dehydrated and infiltrated by the tertiary butyl alcohol-paraffin oil schedule described by Johansen (30, p. 130) and embedded in 56°-58° C Tissuemat.

The procedure outlined by Herridge (18, p. 10-11) was followed in sectioning and staining this material.

THE HOLLY SCAB DISEASE

Holly scab in the field may show considerable variation (Figures 3 and 4). The color of the spots may range from grey to dark red. Spots may be discrete and separate, or aggregated to form larger spots up to 10 mm in diameter. This range of symptom expression has provoked such descriptive names as "blister spot", "pimple spot", "red leaf spot", "corky spot", and "scab".

Holly scab frequently begins as small dark spots around the stomata on the current years leaves. By fall the small dark spots form irregular aggregates and become swollen and blister-like. Often the spots are visible and swollen on both leaf surfaces but more commonly spotting is limited to the lower leaf surface. On the second and third year leaves spotting usually is somewhat larger and more deeply colored.

When sections of leaves affected with holly scab are examined under the microscope extreme cellular proliferation and hypertrophy of the mesophyll is evident. The walls of these affected cells are thickened and sometimes fungus hyphae can be observed.

Part of the problem in studying this condition was the variation in holly scab and early confusion with a number of somewhat similar spots caused by other agents. It was not known if there was one or more than one kind of leaf spot present and thus possibly more than

one causal agent involved. With this in mind, experiments were designed to determine the cause of holly scab and the range of symptoms associated with the disease.

Varietal Response to Holly Scab

A great deal of variability in size and color of scab spots was observed on different varieties of holly. In order to compare the relative susceptibility of holly varieties to scab development, observations have been made periodically on the variety planting at the John J. Astor Branch Experiment Station near Astoria.

This planting of Ilex aquifolium was established in 1951 and presently includes 42 varieties. The orchard, which is on a hillside with uniform soil and good drainage, has received a minimum of chemical sprays in order to allow diseases to develop freely and to facilitate evaluation of varietal resistance. When fungicides have been applied, only one tree of each varietal pair was treated.

Results

Table 1 shows the results of observations made on the paired trees. The varietal type is from a tentative classification prepared by Drs. Blaney and Roberts[1] based on pigmentation of the stem.

[1] Blaney, Lawrence T. and A. N. Roberts, Assoc. Prof. & Prof. of Horticulture, O. S. U., respectively. Unpublished mimeograph.

Varieties are classified as greenstem (little or no pigmentation), brownstem (moderate pigmentation) and bluestem (highly pigmented). It should be pointed out that the brown stem varieties do have significant pigmentation, and in some listings are grouped with the bluestem varieties. Varieties in the plot showing a significant degree of leaf spotting but not included in Table 1 were: Teufel Variegated, Moyer Male, Beauty Spra, Teufel Seedling, Moyer's Boutonnier, Dr. Huckleberry 25, Oregon Select, Leach 600, Bodley Seedling, and Coleman 34.

Varieties with more pigmentation in the stems also tend to form more pigmentation in the leaf spot following infection with the holly scab organism. The greenstem varieties although not resistant do not discolor as severely and therefore would be more desirable for planting stock in areas where the holly scab is a potential threat.

The information presented here indicates that under similar environmental conditions trees of the same age but of different variety may vary widely in their reaction to the holly scab organism. All varieties appear to be susceptible but the Brownell Special is by far the most sensitive to the holly scab fungus and should be used when possible for inoculation and other experimental studies of this disease.

Table 1. Relative severity of holly scab on trees in the Astoria variety planting.

Variety	Holly Type	Scab Development			
		Prevalence	Size	Side of Leaf	Pigmentation
Rederly (Bailey's Pride)	Brown	Moderate	Large	Both	Heavy
Brownell Special	Brown	Severe	Large	Both	Heavy
Escort Male	Green	Severe	Large	Both	Heavy
Duruz	Brown	Severe	Large	Both	Medium
O. S. U. #1	Brown	Severe	Large	Both	Heavy
Yule Glow	Green	Severe	Medium	Lower*	Medium
Besse Selection		Slight	Small	Lower*	Slight
Early Commercial	Brown	Moderate	Large	Both	Medium
Bodley		Slight	Medium	Lower	Slight
Mrs. Pilkington	Green	Slight	Medium	Both	Medium
Dr. Huckleberry	Green	Severe	Medium	Both	Medium
Bleeg Long Spray	Green	Slight	Medium	Both	Medium
Teufel Green Stem (Curley)	Green	Severe	Medium	Both	Slight

* Not as prevalent on the top as on the bottom.

Figure 2. Holly scab on different varieties of English holly from Astoria variety planting. (a) Rederly. (b) Bodley. (c) Teufel Greenstem. (d) O. S. U. 1. (x 1)

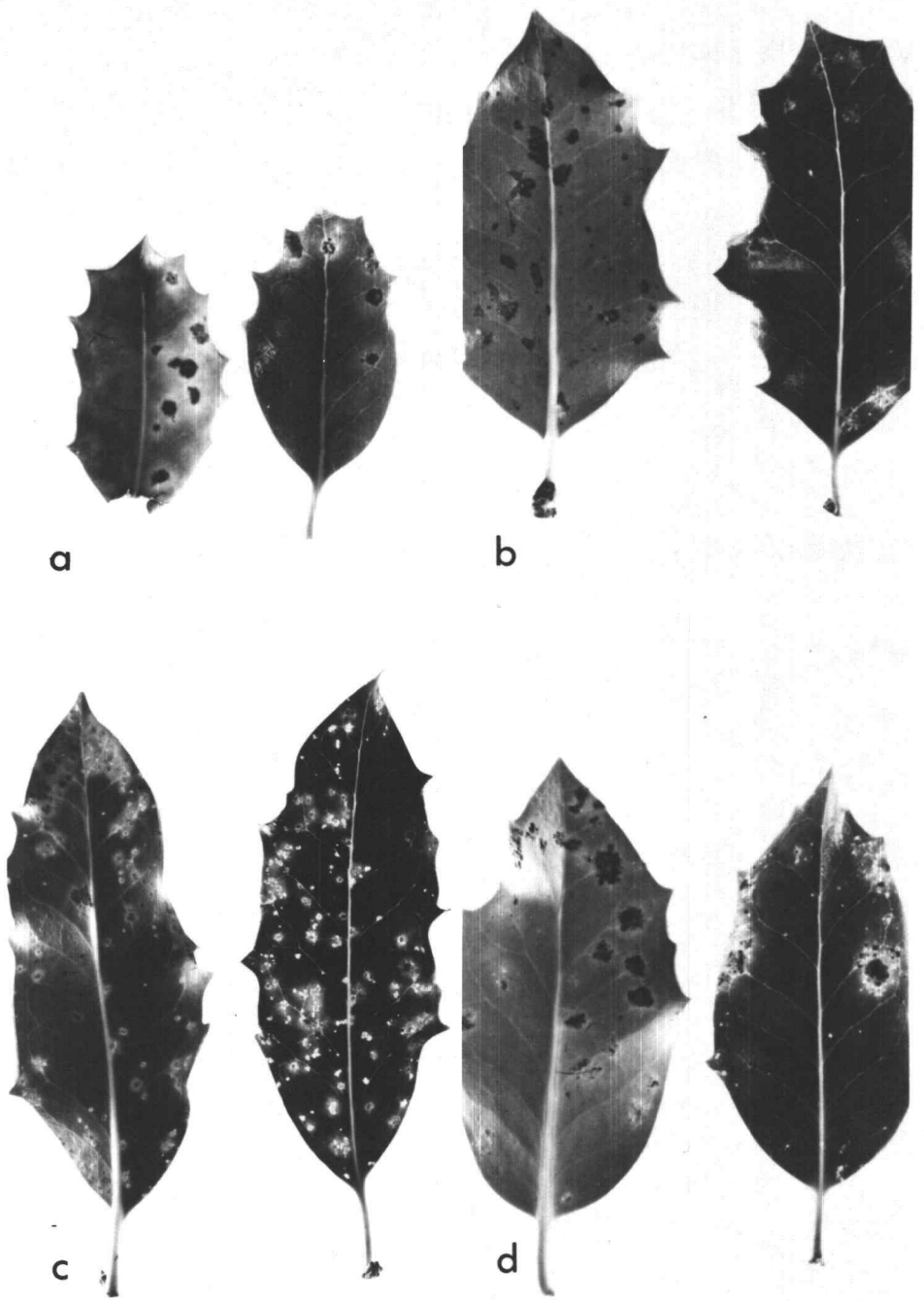
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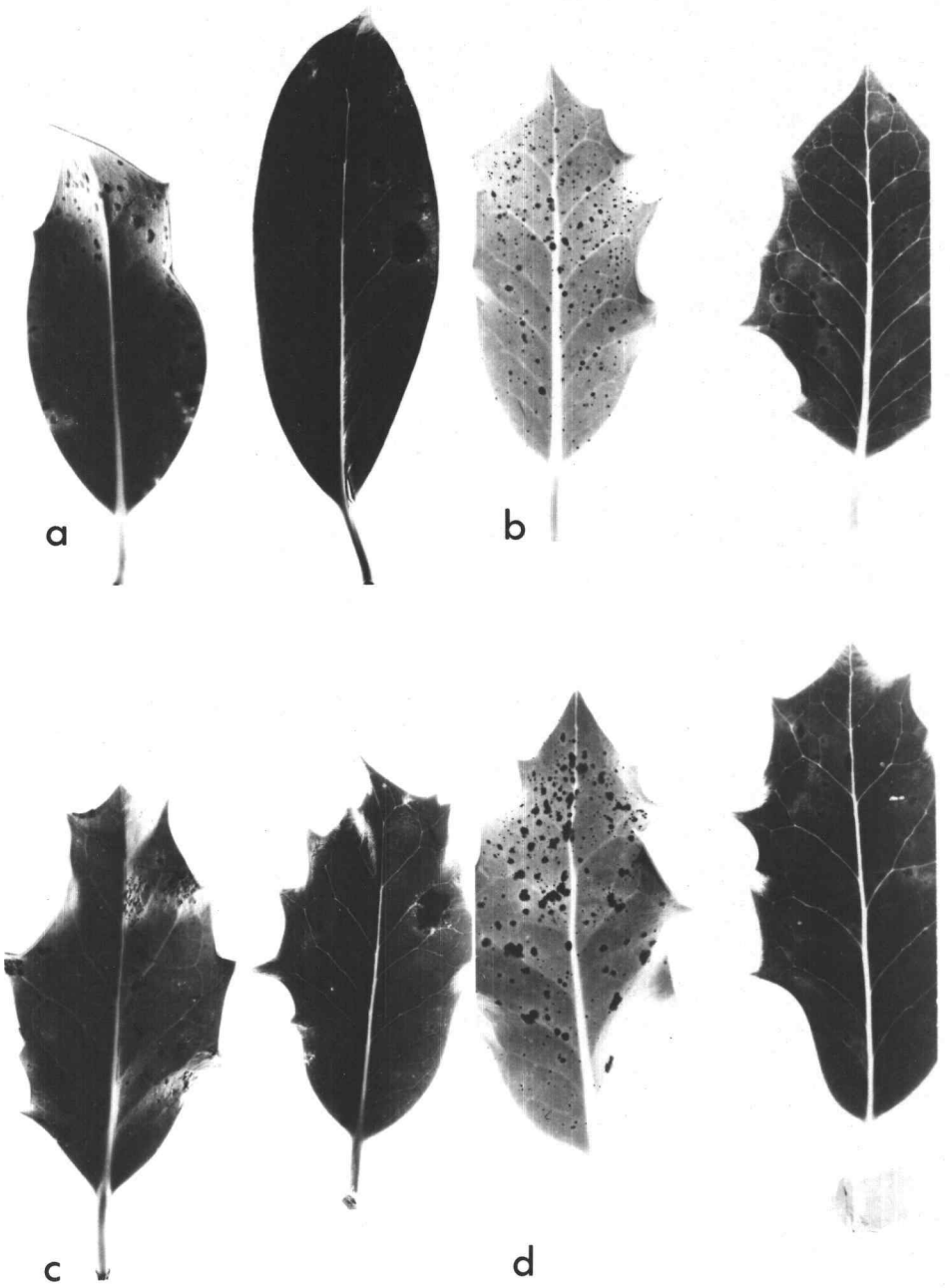
Figure 3. Holly scab on different varieties of English holly from Astoria variety planting. (a) Early Commercial. (x 1/2) (b) Besse Selection. (c) Yule Glow. (d) Huckleberry. (b, c, d x 1)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Leaf Retention

One major undesirable effect of the holly leaf spot disease caused by Phytophthora ilicis is the defoliation that results from the production of ethylene in diseased tissue (6, 7, 8). In a general summary of holly diseases, Buddenhagen (5) observed that there appeared to be no premature leaf drop associated with the holly scab condition. Herridge (18, 19) did not refer to leaf retention in relation to holly scab. The leaves on healthy plants usually will remain on the trees for at least two years and in many cases three years. Because of the importance of leaf abscission to the holly industry, observations were made on both naturally and artificially infected plants of the variety Brownell Special to see if holly scab caused premature leaf abscission.

Natural Infection

Observations of the young trees used in a mineral nutrition experiment revealed no increase in leaf abscission due to the presence of the leaf spot. A high percentage of small trees that were placed in the field in June of 1962 developed severe leaf spotting by September 1962. These plants were returned to the greenhouse at that time and held until 1964. Frequent observations were made and in no case was there a significant dropping of infected leaves over a

period extending to March 1964. At the end of the two year period, the original leaves with the original leaf spots were still on the trees.

Artificial Infection

One group of test plants inoculated in November 1962 showed typical leaf spotting symptoms by January 1963. Uninoculated leaves did not develop spots but the original spots progressively enlarged to sizes larger than those generally found in the field (Figure 5a & b). Subsequent new growth in 1963 was not affected with the leaf spotting condition. Diseased leaves remained on the plants during the second growing season.

In a second group of plants, inoculated in August 1963, severe spotting was observed by October. These spots were extremely large, up to 20 mm in diameter, but the leaves otherwise were normal and remained on the plants.

These observations of naturally and artificially infected leaves support Buddenhagen's original observations that the holly scab condition as we now know it does not stimulate premature leaf abscission.

THE ROLE OF MINERAL NUTRITION IN SCAB DEVELOPMENT

From the work of Roberts et al. (39, 40), boron deficiency is known to produce a characteristic type of red leaf spotting in holly (Figure 1b, c, d). Although the evidence was strong that holly scab was a response to a pathogen, earlier attempts by others and the preliminary efforts in this investigation failed to consistently associate an organism with the holly scab condition. Therefore, an experiment was designed to determine whether or not a nutritional deficiency might be the principal cause of holly scab, or might be prerequisite to infection by a pathogenic organism and/or development of scab. Trials were established in such a manner as to expose nutritionally deficient experimental plants to the best possible source of natural inoculum.

The plants used in this experiment were three-year-old rooted cuttings that had been grown under conditions of low fertility and were only about six to eight inches in height. No fertilizer was added to these plants during the three year period. These cuttings were all of the highly susceptible variety Brownell Special and were taken from the Brownell Clackamas River planting southeast of Portland, Oregon. The rooted cuttings were planted in a 1:1 mixture of sand and peat moss in number ten tin cans that had been coated with tar, and grown in the greenhouse for a period of three weeks without the

addition of fertilizer. On may 6, 1962, and at least every week after this for three months, the trees were watered with various mixtures of a modified Hoagland's solution to produce the nutrient deficiencies desired for these trials. The following treatments were designated as:

1. Complete, no deficiency
2. Boron deficient
3. Nitrogen deficient
4. Boron and Nitrogen deficient
5. Calcium deficient
6. Calcium and Nitrogen deficient
7. Boron and Calcium deficient
8. Half level Nitrogen

Eight different stock solutions were prepared and used to mix the various modified Hoagland's mixtures that were applied to the trees. In order to make a gallon of nutrient solution for each prescribed treatment, the combinations of stock solutions indicated in Table 2 were used. The number of treatment is designated above each column. Each solution was mixed by reading down the column in Table 2 and measuring out the prescribed amount of each stock solution into four liters of water.

Table 2. Stock solutions used to prepare deficient nutrient mixtures for application to host plants.

Stock Solutions	Treatments							
	1	2	3	4	5	6	7	8
245 g/l KH_2PO_4	20	20	20	20	20	20	20	20
122.5 g/l $Ca(NO_3)_2 \cdot 4H_2O$	20	20						
370 g/l $MgSO_4 \cdot 7H_2O$	20	20	20	20	20	20	20	20
85 g/l $NaNO_3$					20		20	20
105 g/l $CaCl_2 \cdot 6H_2O$			20	20				20
Minor Elements Complete	4		4		4	4		4
Minor Elements Less Boron		4		4			4	
FeEDTA 1 g/l	20	20	20	20	20	20	20	20

In all cases there were 15 trees per treatment. The trees were randomly divided into five groups containing three trees of each treatment and each group was placed at a different location in the experimental holly orchard at the John J. Astor Experiment Station near Astoria. A second lot of ten trees with complete fertilizer were kept isolated in the greenhouse at Corvallis as a check on whether or not trees at Astoria became infected with inoculum from that area. The test trees were placed in the field the first week of June 1962 and left there until the first week in October when they were returned to

the greenhouse in Corvallis. Observations of the incidence and severity of spots on each tree and the general condition of the plants are recorded in Table 3.

All trees exposed to inoculum in the holly orchard showed leaf spotting with variations apparently limited by the vigor of the host plant (Figure 4); the more vigorous the host plant the larger and more colored were the leaf spots. The most densely colored were the leaf spots on the boron and calcium deficient and the half-level nitrogen plants. All plants were severely effected except the calcium and nitrogen deficient ones. The non-deficient plants did have larger spots. The condition of the trees remained generally the same until October 1963 except that those plants to which no boron was added showed distinct boron deficiency symptoms and new growth on all treatments showed little or no leaf spotting.

These data indicate that mineral deficiency is not the cause of holly scab and in fact has no significant effect on scab development. The fact that scab did not develop on trees kept in the greenhouse but did develop profusely by October on trees placed in a holly orchard during June, July and August supports the concept that scab is caused by a pathogenic organism and indicates the presence of inoculum relatively early in the summer in the Astoria region.

Table 3. The effect of mineral nutrition and exposure to possible field inoculum on development of holly scab.

Treatment	Plant Condition	Leaf Spotting		
		Prevalence	Size in mm	Color
Test Trees Exposed to Field Conditions				
1. No deficiency	Extremely good growth & color	Severe	7 -- 10	Grey
2. Boron deficient*	Good growth & color	Severe	2 -- 3	Grey
3. Nitrogen deficient	Poor growth & somewhat yellow	Severe	1 -- 2 some 5	Grey to brown
4. Boron & Nitrogen deficient	Very poor growth & quite yellow	Severe	1	Grey to brown
5. Calcium deficient	Good growth & color	Severe	1 some up to 3	Grey
6. Calcium & Nitrogen deficient	Extremely poor growth & quite yellow	Moderate	1 some up	Grey
7. Boron & Calcium deficient	Moderate growth & color	Severe	3 -- 6	Distinct purpling
8. 1/2 level of Nitrogen	Good growth & color	Severe	2 -- 5	Distinct purpling
Test Trees Not Exposed to Field Conditions				
9. No deficiency	Good growth & color	-----	No Leaf Spotting	-----

* Distinct boron deficiency leaf spotting was observed by October 1963.

Figure 4. Holly scab on plantings of varying nutrient levels that were exposed to natural infection in Astoria variety plots. (1) No deficiency (Control). (2) Boron deficient. (3) Nitrogen deficient. (4) Boron and Nitrogen deficient. (C) Control - not exposed to field inoculum (spots are white-wash) (5) Calcium deficient. (6) Calcium and Nitrogen deficient. (7) Boron and Calcium deficient. (8) Half level of Nitrogen.


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THE ASSOCIATION OF FUNGI WITH HOLLY SCAB

In an effort to determine the cause of holly scab, extensive isolations were made from different holly varieties collected in various parts of the state. During the summer and fall of 1961, 111 fungus isolates were isolated from English holly affected with holly scab. The methods of collection and isolation were as follows:

Samples of leaves were collected in the field and types of spotting and severity were recorded. Leaves were then placed in plastic bags for transport to the laboratory. Unless isolations were attempted immediately, the leaves were stored at 4^{o} C in the plastic bags until such time as the isolations could be attempted.

In routine isolations, the leaves were washed in running tap water for one-half hour and sections containing individual leaf spots were cut from the leaves. The size of these sections varied somewhat depending upon the size and density of the leaf spot. Usually, the sections were ten mm square or larger. The sections were surface sterilized in a five percent Clorox solution for five minutes and rinsed in sterile water. One section at a time was then subdivided into approximately two mm square sections with flamed forceps and scalpel. These smaller segments of the leaf spot were placed in petri dishes containing two percent potato dextrose agar or water agar and held at room temperature and checked frequently for any apparent fungus growth.

Once fungus growth was observed, hyphal transfers were made to PDA plates and allowed to grow until sufficient material was available for examination and identification. In most cases, identification was made with the aid of either Clements and Shear's "Genera of Fungi" (9), or H. L. Barnett's "Illustrated Genera of Imperfect Fungi" (3). After a fungus had been observed and studied in culture, mass transfers were made to PDA slants in test tubes and stored at 4° C.

Inoculations

None of the isolates identified had been proven previously to be pathogenic on holly. Thus, inoculation trials were initiated as a method of screening the many isolates.

In all of the inoculation trials, two-year-old trees of the same comparative vigor were used.

Prior to inoculation, all trees were placed in a continuous mist chamber at 25° C. Then small squares, 5x5 mm, were removed from petri dish cultures and placed on the lower surface of the leaves on the test plants.

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

off. In this manner an extremely slow growing fungus was given a nutrient and moisture source until it could become established.

Inoculations were first attempted August 22, 1961. Nine different isolates and a control of uninfested agar discs were used. After nine months no symptoms had developed.

The next inoculations were attempted October 30, 1961. Eighteen different isolates and agar disc controls were used. None of these caused leaf spotting except isolate 70 which showed distinct holly scab symptoms after a three month period. This leaf spotting was typical of that found under field conditions but more severe (Figure 5a, b). This isolate was tentatively identified at that time as Cuticularia ilicis Ducomet. The source of this material was a collection of Rederly from the Richen farm near Sherwood, Oregon, on June 9, 1961. The original collection showed severe blister leaf spot symptoms as well as boron deficiency symptoms.

Subsequent inoculations were made in July 1963, using this isolate and the same method of inoculation. In these trials, typical leaf spotting consistently followed and was well advanced by October 1963.

Of the 27 isolates used in these inoculation studies, all were negative with the one exception of isolate 70, Some of the fungi that gave negative results were of the genera Stemphylium, Fumago, Alternaria, Penicillium, and Cladosporium, while others were not

identified.

Artificially infected leaves were collected and divided into two groups. One group of leaves was used for histological examination and the second group was used for isolations in which the fungus was recovered. The method of reisolation was the same as that described for the initial isolations.

Histological Observations of Inoculated Leaves

Herridge's (18, 19) studies showed that the blister-like swelling were due to cellular proliferation and hypertrophy within the infected area of the leaf mesophyll. Also fungus hyphae were reported to be present in this abnormal tissue.

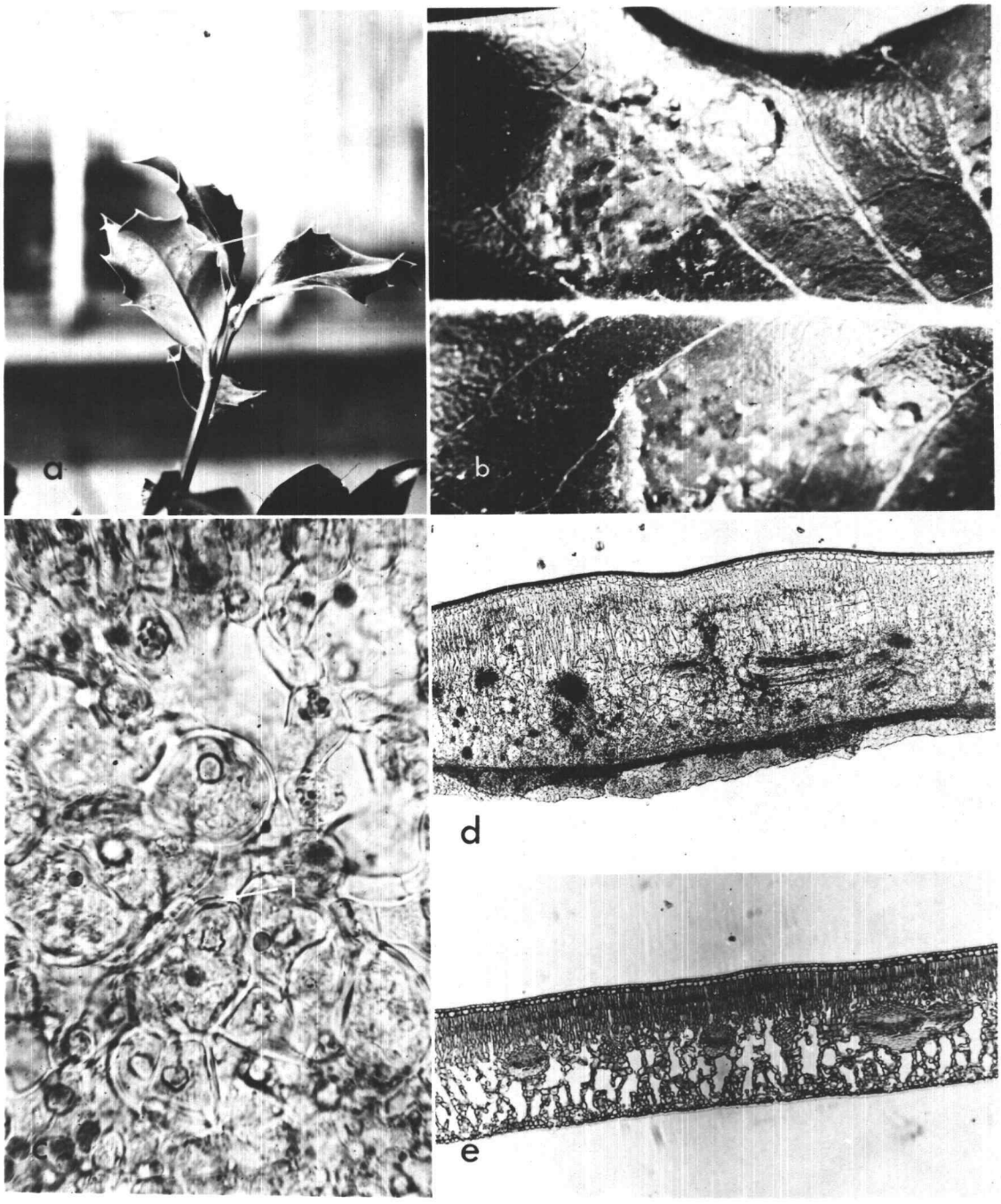
In the material inoculated with isolate 70, the same phenomena were observed (Figure 5). These included:

1. the presence of hyphae in the abnormal tissue,
2. significant deposition of resinous materials and accompanying necrosis,
3. extremely proliferated spongy mesophyll and resulting obliteration of intercellular spaces,
4. degenerate or absent chloroplasts in infected tissue.
5. an intact upper and lower epidermal layer.

No sporulation was observed in any of the innoculated material.

This information supports and confirms that the holly scab condition is fungus induced. It also shows that the blister-like spotting is due to a hypertrophy and proliferation of the leaf mesophyll as described by Herridge.

Figure 5. Holly scab development on inoculated leaves. (a) Raised, swollen, translucent spots formed on both abaxial and adaxial leaf surfaces. (1/2 x) (b) Raised, swollen, translucent spots formed on adaxial leaf surface. (3x) (c) Highly enlarged section of spongy mesophyll showing cellular hypertrophy, proliferation and thickened cell walls, l-fungus hyphae. (400x) (d) Cross section of inoculated leaf showing cellular proliferation, hypertrophy and obliteration of intercellular spaces. (28x) Cross section of normal leaf showing abunate intercellular spaces in spongy mesophyll and normal cellular composition. (28x)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SOME PHYSIOLOGICAL ASPECTS OF THE HOLLY SCAB FUNGUS

Examination of isolate 70 led to the conclusion that it had not previously been described as a pathogen on English holly. At this time, no sporulating form of the organism had been observed and described, so it was considered to be a member of the Mycelia Sterilia and tentatively identified as Cuticularia ilicis (9, p. 232; 42, vol. 22, p. 1502). Even though isolate 70 and Cuticularia were quite similar this was a tentative diagnosis because Cuticularia was not well defined.

Therefore, in order to properly identify and describe the fungus, attempts were made to stimulate sporulation of the fungus in culture. The method of doing this was to subject the organism to variations of temperature, pH, and nutrition and exposure to near ultraviolet irradiation. While attempting to bring about sporulation, vegetative growth optimums under these different conditions could be determined. It was also hoped that these observations would contribute to a fuller understanding of the disease syndrome.

Temperature

Determination of the optimum temperature for vegetative growth and the effect of temperature on the sporulation of the fungus was studied, starting with cultures 60 mm in diameter. From the

margin of the stock colony, inoculum discs were cut with a five mm cork borer and transferred to petri dishes containing strep-PDA. Thus, all initial inoculum units were of the same size and age. The cultures were allowed to grow at room temperature for three days and then placed in temperature cabinets adjusted to 5, 10, 15, 20, 25, and 30° C, ± one degree. Each temperature treatment was replicated five times and the experiment repeated.

Colony diameter was recorded once a day for a period of six days. Two measurements were taken across each colony at right angles to one another and always at the same position.

Results

The vegetative growth of the fungus is expressed in average accumulative increases (mm) of colony diameter. This is shown in Figure 6. The fungus grew well at temperatures ranging from 15 to 25° C, moderately well at 10° C, slowly at 5° C, and did not grow at 30° C. This indicates that temperature probably would not be a limiting factor in Oregon in development of holly scab. Instead, temperatures would be favorable for growth of the organism during much of the year.

This experiment failed to show a temperature effect upon the sporulation of the fungus when grown on strep-PDA.

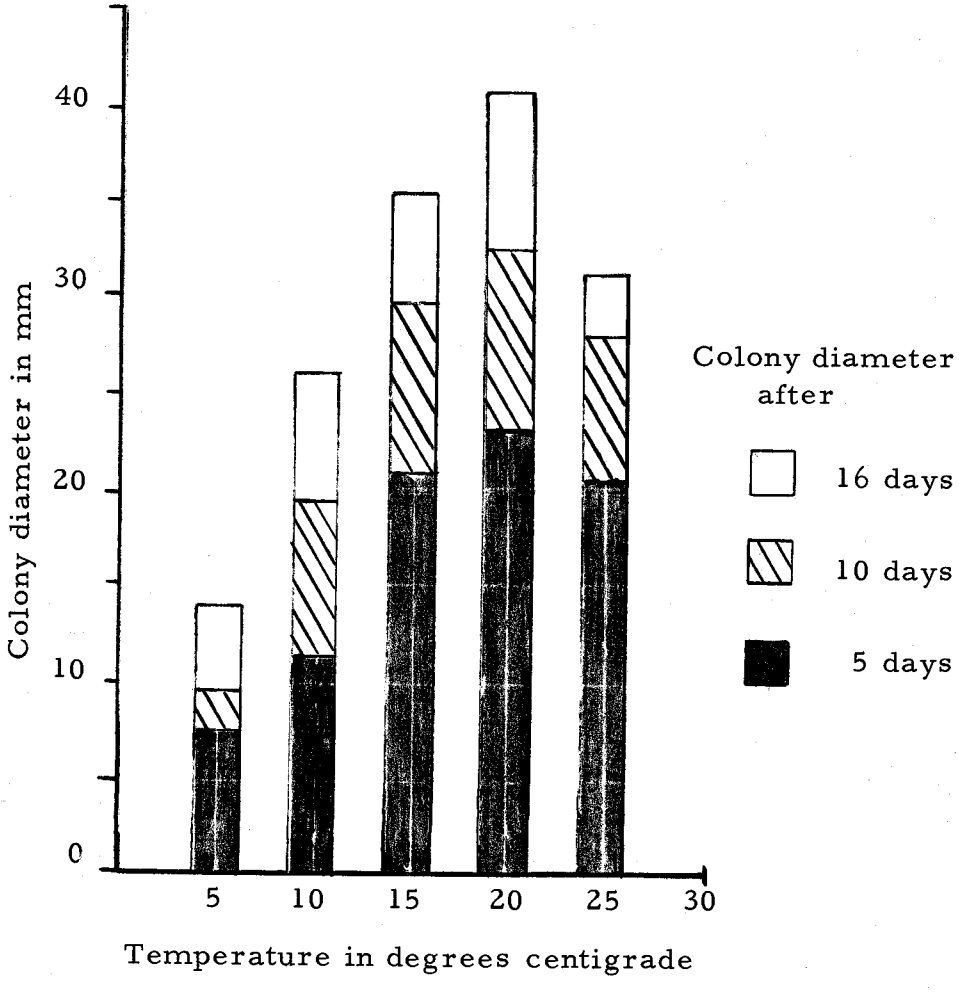


Figure 6. Effect of temperature on vegetative growth of the holly scab fungus.

Fungus Response to Variation in pH

In order to fully understand and study the morphology of the organism, the effect of various pH levels upon the vegetative growth and sporulation of the fungus was examined.

Methods

The basic semisynthetic medium used here is that which was described in the general materials and methods section except that K_2HPO_4 was used instead of KH_2PO_4.

A citrate phosphate buffer was used at pH 3, 4, 5, 6, and 7, while a boric acid-borax buffer was used at pH 8 and 9. The basic formulation of these buffers was taken from Methods in Enzymology (15) and modified as outlined below.

Stock solution for Citrate Phosphate Buffer -

A: 0.2 M solution of citric acid (19.21 gm/500 ml water).

B: 0.45 M solution of dibasic sodium phosphate (53.65 gm of $Na_2HPO_4 \cdot 7H_2O$ or 71.7 gm of $Na_2HPO_4 \cdot 12H_2O$/ 500 ml H_2O).

Schedule A	B	Desired pH
39.8*	10.2	3
30.7	19.3	4
24.3	25.7	5
17.9	32.1	6
6.5	43.6	7

* Volumetric measurements in milliliters

The 50 ml of buffer was then added to 450 ml of medium and necessary minor adjustments were made with stock solution as determined with a Beckman model 72 pH meter.

Stock solution for Boric Acid-Borax Buffer -

A: 0.42 M solution of boric acid (12.4 gm. 500 ml water).

B: 1.0 M solution of borax (19.05 gm/500 ml or 1.2 M solution in terms of sodium borate).

Schedule -- 50 ml of A and x ml of B were added to 400 ml of culture media and then adjusted to the desired pH with stock solution as determined with a Beckman as above.

The buffered medium was then filter-sterilized and dispensed as described in the general methods section. One hundred flasks of this medium were prepared and held for several days at room temperature before they were inoculated. No contamination developed. Following inoculation, the flasks were held at room temperature.

In preliminary studies the fungus was shown to grow well in liquid culture even without extensive shaking. Therefore, the flasks were shaken only twice a day.

The pH was recorded both initially and at the time of harvest. Two harvests were made, the first nine days after inoculation and the second 13 days after inoculation.

The method of harvest was as follows:

1. Fiber glass filter paper (Whatman GF/A 7.0 cms) was dried in a forced-draft oven at 100° C for 12 hours, then weighed on a Mettler analytical balance.

2. Fungus cultures were washed onto the previously weighed filter paper, dried in the oven at 100° C for 24 hours, and then allowed to cool in a dessicator.

3. Total weight was then recorded and increase in mycelium weight computed.

4. Each pH level treatment was replicated five times.

Results

The fungus markedly changed the pH of several media after 13 days incubation but did not do so after nine days.

Initial pH	pH at 9 days[1]	pH at 13 days
3	3	3.14
4	3.96	6.22*
5	5.3	6.92
6	6.5	7.05
7	7.15	7.43
8	7.9	8.0

[1] average pH value

* extreme variation here in pH at harvest; 5.1, 7.2, 7.1, 7.2, and 4.5

The increase in dry weight after nine days was significantly greater at pH 4. But, after 13 days (after buffering had broken down in some cases) greatest growth increases occurred at initial pH 4, 5, and 6 (Figure 7).

No sporulation was observed at any pH. The explanation of this

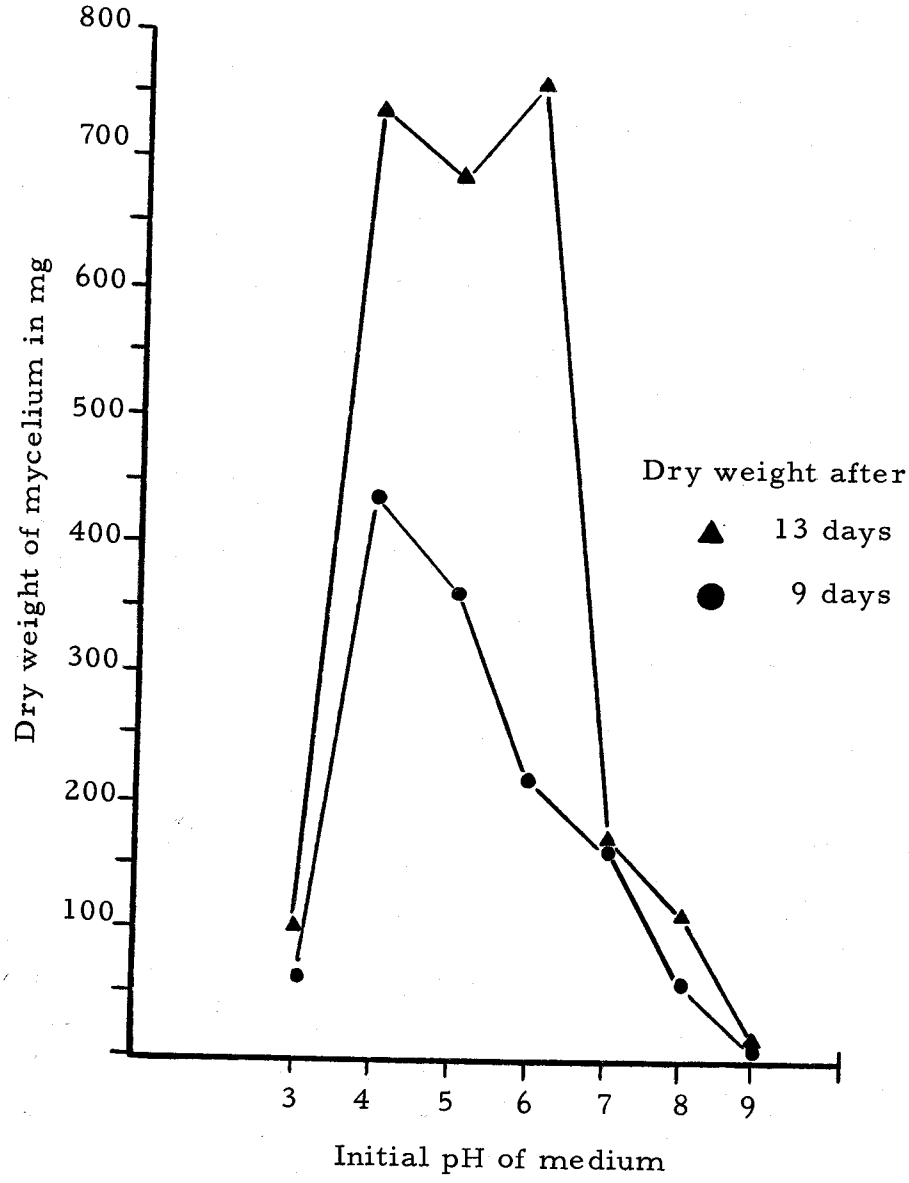


Figure 7. Effect of pH on vegetative growth of the holly scab fungus.

became clear upon subsequent experiments in which it was observed that asparagine (which was used in these media) inhibits the sporulation of this fungus.

The holly scab pathogen grew best in media at an initial pH of 4 but also grew well over a pH range from 4 to 6, grew slowly at 3, 7, and 8, and failed to grow measurably at pH 9.

Effect of Nitrogen Source on Sporulation

In experimental work using the basal medium with asparagine as the nitrogen source, no sporulation was observed. Asparagine has been reported to exert an inhibitory effect on the sporulation of some fungi (21, p. 6). Therefore, it seemed advisable to compare media containing asparagine to media containing a different nitrogen source, preferably inorganic.

The basal semisynthetic medium was used as previously described, with the exception that it was prepared without the nitrogen source. Once the medium was prepared, it was divided into two portions. To one portion one gram of asparagine was added, while to the other portion one gram of KNO_3 was added.

The pH was adjusted to 7 with the modified citrate-phosphate buffer. Five flasks of each medium were prepared by filter-sterilizing and dispensing as described in the general methods section. All flasks were allowed to stand several days before inoculation

to insure lack of contamination. Flasks were inoculated by mass transfers, held at room temperature and observed over a 20 day period.

Results

None of the media with asparagine as the nitrogen source showed signs of sporulation after a 20 day period. All replicate media with KNO_3 as a nitrogen source had sporulated over an 11 to 15 day period, with most of them sporulating at 15 days. After 20 days, the sporulation on the KNO_3 medium was quite dense.

Near-ultraviolet Stimulation of Sporulation

Recent work (23) has demonstrated that many fungi which do not sporulate readily under normal light will sporulate readily and profusely under exposure to near-ultraviolet light (U. V.). An experiment was designed to determine if U. V. would stimulate sporulation of the holly scab fungus.

Thirty plates of strep-PDA were inoculated with 5mm agar-mycelium discs, incubated in continuous darkness at room temperature for five days, then randomly divided into three groups of ten plates each. Each of the three groups was subjected to one of the following treatments:

1. kept in continuous darkness as a control,

2. exposed to continuous white light from Ken-Rad 40W cool white fluorescent lamps,

3. exposed to continuous near-ultraviolet from Sylvania, Blacklite-Blue, F 40 BLB lamps.

Plates were held for 30 days and then examined as to color, type of mycelium and presence or absence of sporulation. The effect of the various treatments is recorded in Table 4. All plates under U. V. exposure had shown some degree of sporulation between 17 and 21 days and extensive sporulation after 30 days. None of the cultures in dark or in white light had sporulated after a 30 day period.

Table 4. Effect of near-ultraviolet light on the growth and sporulation of the holly scab fungus.

	Colony Characteristics after 30 Days		
Treatment	Mycelium Color	Dimorphic Mycelium	Sporulation
Control (In the dark)	light brown	absent	absent
White light	dark brown	present	absent
Near-ultraviolet light	dark brown	present	present

Sporulation of the holly scab fungus is stimulated and enhanced by prolonged exposure to near-ultraviolet light, but is inhibited by continuous darkness and white light.

Pigmentation and formation of dimorphic mycelium is stimulated by both white and near-ultraviolet light but not darkness. This dimorphism is expressed by the occurrence of young mycelium that is generally hyaline, quite uniform in diameter and somewhat irregularly septate, while the older form is dark brown, very irregular in diameter and highly septate.

IDENTIFICATION AND DESCRIPTION OF THE HOLLY SCAB FUNGUS

The preceding work demonstrated conclusively that holly scab is a fungus-induced condition. This accomplished, the final step then is to characterize and identify this fungus. Inasmuch as no fruiting bodies have ever been associated with holly scab on leaves in the field it is necessary to resort to a description of the fungus as it appears in culture.

The fungus material examined was grown on strep-PDA media and sporulation was induced by exposure to near-ultraviolet light.

Material to be examined was mounted in a drop of water and a small amount of sodium carboxy methyl cellulose (NaCMC) was added to immobilize the spores to facilitate measurements (29). A 12 percent gel of NaCMC has a refractive index of 1.3418 (29) and does not significantly alter the appearance of the spores or other material.

All measurements were made with an ocular micrometer but four different microscope-ocular combinations and camera lucida measurements were used in order to insure accuracy. Where color was interpreted or given, the standardized Horticultural Colour Chart (4) was used as the standard reference.

Results

The fungus grows very slowly on strep-PDA producing colonies that when young have a greyish sung green (4, vol. 2, p. 195) color but turn to a darker grey carnation green (4, vol. 2, p. 194) color with age. The mycelial growth is very prostrate on the surface of the medium and is somewhat tomentose to subtomentose in appearance. Generally the growth is uniform and the margins of the colonies are smooth or even. With aging, the medium becomes quite discolored, turning a dense mars orange (4, vol. 2, p. 104) color.

Mycelium of young colonies is hyaline, somewhat irregularly septate and branched and the cells are generally uniform in diameter. With age, another mycelial form develops which is highly septate, irregular and very opaque and becomes dark brown in color. Frequently the more highly septate, irregular and opaque mycelium tends to clump and form multi-filament strands.

The fungus forms densely opaque pycnida from the older mycelium. These pycnidia vary in size from 75u to 200u. A distinct feature of these pycnidia is their lack of an ostiole. Pycnidia are usually oval but occasionally are pyriform. The young pycnidia are very membraneous and of uniform density throughout. With maturity they become dense and dark. Pycnidia are usually formed separately rather than in clumps or aggregates.

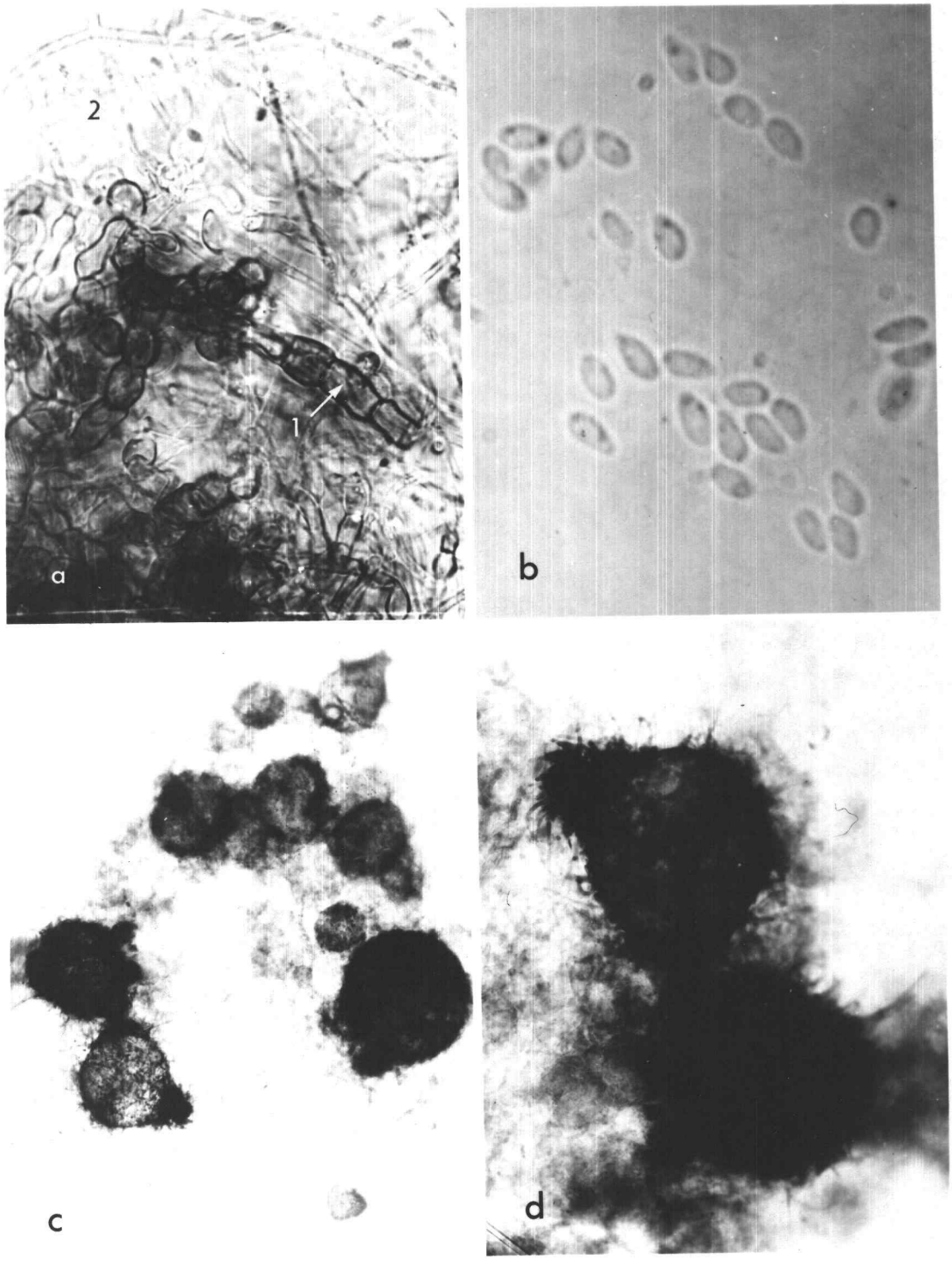
Spores are single celled, hyaline, ovoid to oblong, 5-7 x 2-4 microns. They are immersed in a hyaline mucous that is readily dissolved in water. Many of these spores are distinctly guttulate, having one or two oil droplets.

The above characteristics indicated that isolate 70 was either Phoma or Phyllostica. Isolate 70 did not agree in all aspects, however, with either of these two genera.

Therefore, suitable living fungus material was sent to Dr. B. C. Sutton of the Commonwealth Mycological Institute who specializes in the Sphaeropsidales. Dr. Sutton concluded that isolate 70 definitely did not belong to either Phyllostica or Phoma but was a Sclerophoma, being similar to S. pithyophila (Cda) Hohn. of which type material is deposited at the institute. He, therefore, placed the isolate under accession number IMI 107649 and designated it as a Sclerophoma sp.

At this time there are no known available collections of Sclerophoma in this country for comparisons with the holly Sclerophoma. Due to the definite difference in hosts, S. pithyophila being reported only on coniferous material, it is likely that the holly Sclerophoma is an undescribed species. Attempts to obtain material deposited at various English and European herbaria are underway and, once this material is available, inoculation trials and morphological studies will be initiated to clarify the species status of the holly Sclerophoma.

Figure 8. Holly scab fungus in culture. (a) Sclerophoma showing dimorphic mycelium 1-older, irregular, darker form, 2-young uniform, not as frequently septate form. (950x) (b) Sclerophoma spores showing ovoid-oblong shape and hyaline color. (1000x) (c) Pycnidia showing both circular and pyriform shapes. (94x) (d) Pycnidia showing pyriform shape and lack of ostiole. (375x)


2
1
a
b
c
d

CHEMICAL CONTROL TRIALS

Chemical control trials were conducted in 1962 with the dual purpose of attempting to learn more about the nature of the disease, and developing an economically useful control program even though at that time the casual relationships were not fully understood.

Methods

A program of orchard spraying was initiated in October 1962 in the Astoria area where the leaf spotting condition was prevalent. A representative orchard at Knappa belonging to Robert Engbloom was selected for the first trials. This was a marginal orchard with a history of leaf spot. The trees were of the Dutch-English type and approximately 20 years old.

Three replicate blocks of 16 trees each were selected for spray trials. Four chemical treatments within each block were used at three different times, with four trees per treatment in each block. The treatments were as follows:

Spray Materials

1. Lime sulfur 4 gal/100 gal of water.
2. Tri-basic copper sulfate (Microcrop) 2 lbs/100 gal water. *
3. Nabam (Dithane D-14) 2 qts. and 1 lb. $ZnSO_4$/100 gal water.*
4. Control, with no chemical applied.

* Two ounces of Triton B-1956 sticker-spreader was used with the copper and nabam formulations.

Application Time

A. Applied in the fall only.

B. Applied in both fall and spring.

C. Applied in the spring only.

Method of Application

Chemicals were mixed in a 100 gallon sled-mounted spray tank. The tank unit was a fiber-glass shell divided into one 50 gallon compartment and two 25 gallon compartments. Each compartment had its own outlet so spray mixtures could be formulated and used in 25, 50, 75, or 100 gallon quantities. Continuous drive paddle wheels in each of the compartments kept the spray materials in suspension.

The tank agitators and the pump were driven by a three horse power air-cooled engine. The pressure was controlled by a relief valve and all spraying was done at 325 psi with a Spraying Systems 42L Gunjet manual spray gun.

The spray machine was mounted on a pickup to facilitate the positioning of the spraying apparatus so that all trees could be adequately reached with 100 feet of hose. The orchard has been sprayed six times since the initiation of the trial. The dates of application

and the prevailing climatic conditions are shown in Table 5.

Table 5. Prevailing temperature and moisture conditions during Engbloom fungicide trials as recorded at Astoria.

		Temperature*			
Date	Precip-itation	Appli-cation	Monthly Ave.	Normal	Subsequently
10/18/62	Trace	61/45	61/46	61/45	Normal
5/3/63	.58	55/39	62/45	62/45	Normal
6/12/62	0.00	65/43	63/49	65/50	75/44, 76/47
7/10/63	Trace	68/54	67/53	69/53	Normal
10/19/63	.62	60/46	62/48	61/45	Normal
6/24/64	0.00	64/42	62/48	65/50	Normal

* Both the high/low are recorded.

Sampling Method

In sampling the orchard, each tree in a block was examined on all four sides and presence or absence of the fungus or other leaf spotting conditions noted. A shoot about five feet from the ground, usually with five or six recent years leaves, was selected randomly and examined. In all cases, the total number of leaves examined as well as the number of diseased leaves were recorded. Results of the spray trials were read on several different dates.

Results

No information was gained from these trials on the effectiveness of chemical sprays because no holly scab infection occurred (Table 6). Significant data were obtained from the trial, however, on three

other kinds of leaf disfigurations.

A. Copper damage. A leaf spot somewhat raised, circular, white in the center and with a distinct deep red border. Frequently the spot would show through as a red blotch on the upper surface of the leaf (Figure 9a).

B. A nabam associated leaf spot. A leaf spot only observed on the lower surface of the leaf, generally irregular in outline and having a distinct uniform grey color. This spot was somewhat raised but not significantly so (Figure 9a).

C. Purple blotch. This leaf spot varied from small scattered areas to large spots covering a major portion of the leaf surfaces. Generally, the spots appeared on the upper surface of the leaf as deeply pigmented areas with irregular, indefinite margins. The surface was not raised and the pigmentation was not pronounced on the lower side of the leaf (Figure 9b).

There was no spotting in the control block (Table 6) except for the occurrence of the Purple Blotch which was observed in October and then disappeared by the following spring.

These data fully substantiate observations (20) that fixed copper can cause severe leaf spotting. In addition, this kind of spot is more

Table 6. Results of fungicide application trials at Engbloom orchard.

I. 1962-63 Growing Season Application Time	Tribasic Copper Sulfate	Nabam	Lime Sulphur	Control
Fall	Extreme chemical damage leafspot A	Clean	Clean	Clean[1]
Fall & Spring	Extreme chemical damage a leaf spot & defoliation	Severe chemical damage as leaf-spot B	Clean but heavy residue	
Spring	No chemical damage	Severe chemical damage as leaf-spot B	Clean but heavy residue	Clean
II. 1963-64 Growing Season **Application Time**				
Fall	Severe chemical damage as leafspot A	Clean	Clean	Clean
Fall & Spring	Severe chemical damage as leafspot A	Clean-chemical damage only on previous years leaves	Clean-some residue	Clean
Spring	Severe chemical damage as leafspot A	Clean-chemical damage only on previous years leaves	Clean-slight residue	Clean

[1] Purple Blotching appeared during late fall and winter months on everything; refer to Table 7.

fully characterized and was found to develop severely at the concentrations recommended for algae and Phytophthora control on English holly in the Pacific Northwest. Also, a certain amount of defoliation was observed with the fixed copper spray material. The data from this trial provide no additional information as to why copper sprays may be used with no injury in many cases but in others may cause defoliation and/or development of red leaf spots.

The leaf spotting that was associated with nabam occurred only on trees sprayed in the spring of 1963. In the second season there was no damage. This may be accounted for in either of two ways: (a) the amount of chemical applied; only one spring application was used in 1964 in contrast to three applications in the previous spring, so there was more accumulated material on the foliage; or (b) the occurrence of higher temperatures at and immediately following nabam applications in the first spring season.

Because of the severity of the nabam associated injury in 1963 and the failure of nabam to cause similar injury in 1964, this problem is being studied further.

Table 7. Time of occurrence of Purple Blotch leaf spotting.

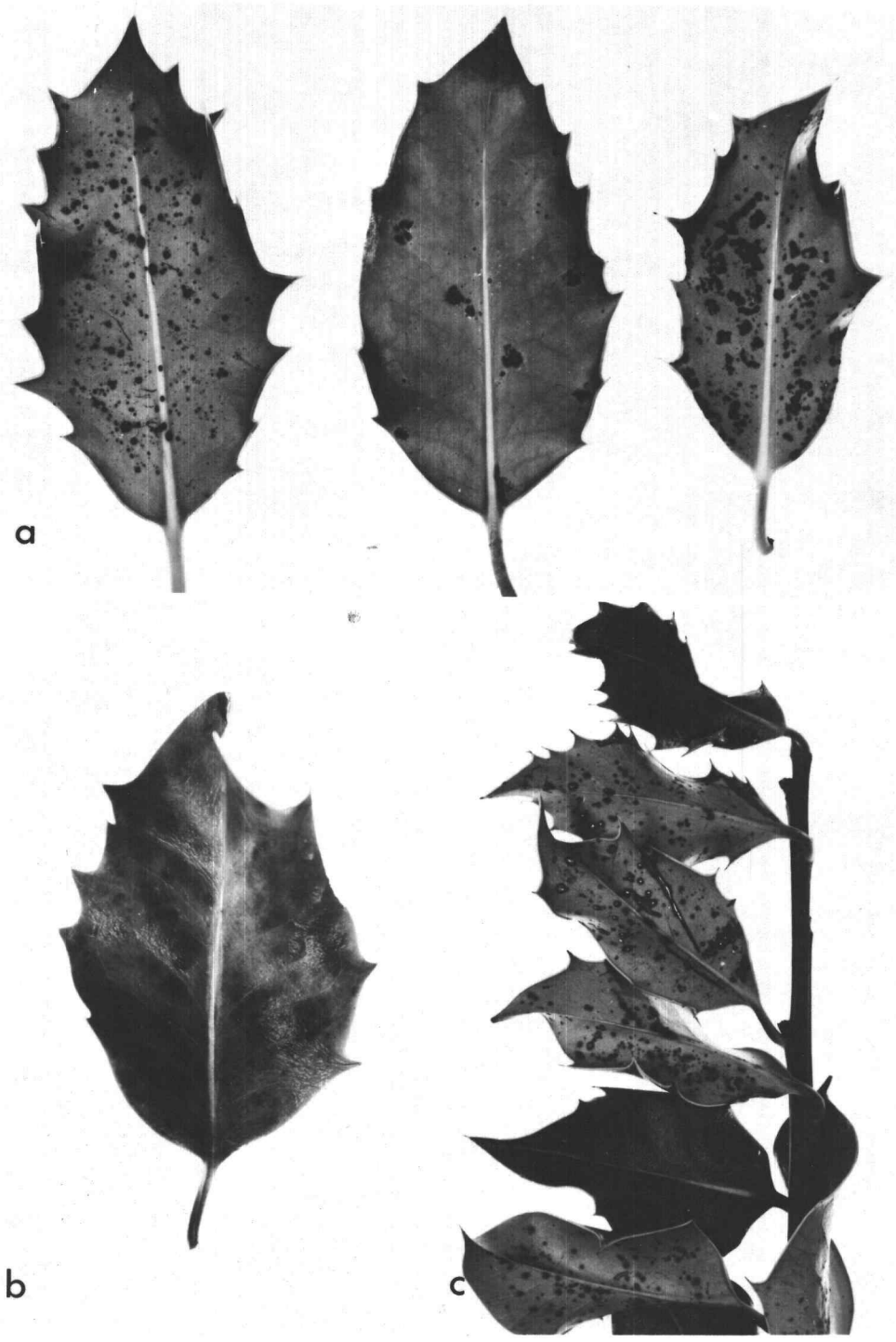
Treatment	1963			1964		
	8/7	9/10	11/30	3/7	6/24	11/14
Control	neg.	neg.	pos.	pos. *	neg.	neg.
Lime Sulfur	neg.	neg.	pos.	pos.	neg.	neg.
Nabam	neg.	neg.	pos.	pos.	neg.	neg.
Tribasic copper sulfate	neg.	neg.	neg.	pos.	neg.	neg.

* Spotting appeared to be more severe on:
a -- shoots with a lot of berries.
b -- lower branches of the trees.

These data indicate that Purple Blotch is not a fungus induced condition because of the loss of excessive pigmentation and reversion to normal leaf appearance with the advent of warm weather and favorable growing conditions; and the widespread occurrence of Purple Blotch irrespective of any chemical spray treatment.

Purple Blotch cannot be associated with any form of spray damage because of its frequent occurrence in the unsprayed blocks. It is therefore more likely related to malnutrition or some form of physiological imbalance that coincides with the late ripening of berries in the fall. This information would then support Guba's hypothesis (17, p. 21) that the purple blotching is considered to be related to malnutrition and the exact circumstances are not clearly understood He further reports that no primary organism can be cultured from these leaf spots. These data would also contradict reports (45, 46) of good control of the blotching conditions with a zineb application in spring and late summer.

Figure 9. Holly leaf spots. (a) From left to right, spotting caused by fixed-copper, holly scab fungus, and nabam. (b) Abnormal pigmentation known as purple blotch. (c) Mechanical damage due to spine punctures showing holes as well as red blister-spot effect. (x1)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DISCUSSION

Holly scab is a disease that can cause serious economic loss to northwest holly growers because of the disfiguring leaf spots that result following infection. The cause of holly scab has been a matter of speculation for some years; physiological deficiencies, chemical toxicity, insect feeding injury and fungal infection have been considered as possible causes. This study indicates that the disease is fungus-induced.

Earlier research determined the cause of several similar types of leaf spot conditions on holly. Most of these are distinct and differ significantly from the swollen, irregular, red, holly scab spots as well as from one another. Mechanical damage usually shows a puncture where the spine from another leaf has penetrated. Midge injury may result in a round pigmented spot but no abnormal cell growth or necrosis can be observed, and frequently the midge can be found on the underside of the leaf. Spots caused by fixed-copper injury are more uniform, circular and frequently have a greyish center. Spots associated with summer usage of nabam are of a uniform grey-brown color and are extremely irregular in size and outline.

Red spots associated with boron deficiency are generally partially concentric and not swollen. In some instances, boron

deficiency is manifested on young leaves as a collapse of the leaf mesophyll. An accumulation of purple pigment, most likely anthocyanin, that has no sharp border or uniform shape and disappears with advent of warm weather and the resumption of vegetative growth is referred to in this thesis as purple blotch. This condition appears to be nutritional.

In seeking the cause of holly scab, more than 100 isolates were isolated from leaf samples collected throughout the Willamette Valley and North Coastal areas of the state between 1961 and 1963. Of these, 28 were ultimately used in inoculation attempts. Only one organism consistently produced leaf spotting typical of the holly scab condition.

This organism initially was identified as Cuticularia ilicis which was reported by Ducomet in 1906 to cause a leaf spot of holly. Subsequent treatment of the fungus, however, resulted in development of fruiting bodies. Attempts to identify the organism repeatedly lead to the genus Phoma. The holly scab fungus did not match well with some of the significant characters of Phoma, however, and was sent to Dr. B. C. Sutton, a specialist in the Sphaeriaceous fungi, at the Commonwealth Mycological Institute in England for additional checking. Sutton tentatively identified the holly scab fungus as a species of Sclerophoma.

Subsequent comparison with Grove's (16, p. 155-157) descriptions of Sclerophoma shows that the holly scab fungus has the generic characteristics of Sclerophoma.

1. Both produce very thick-walled pycnidia.
2. Both have a well developed stromatic tissue of pale cells similar to those of the sclerotium proper.
3. Neither possesses distinct sporophores.
4. Both have a gelatinous matrix in which the spores are embedded.
5. Both have spores that are single-celled, ovoid to oblong and hyaline.
6. Neither has a distinct ostiole.

It is principally on the basis of this last character, the lack of a distinct ostiole, that the holly scab fungus is placed in the genus Sclerophoma rather than Phoma which it resembles in many respects.

Three species of Sclerophoma have been described (16, p. 155-157), none of which have been proven experimentally to be plant parasites or reported on Ilex. At present, no material of any of the described Sclerophoma species is available for inoculation trials or morphological comparison with the holly Sclerophoma so comparisons must be based on descriptions of the other species listed in Grove (16, p. 155-157). The holly pathogen most closely resembles S. pithyophila except that pycnidial measurements do not

compare closely. Leach (23) 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 the pycnidia would hardly seem an adequate basis to justify a species difference.

The holly scab fungus is the first demonstrated plant pathogenic Sclerophoma, however, and on this basis and morphological differences may be a new species. It would appear at this time, however, to be more advisable to make careful morphological and pathogenicity comparisons of the holly Sclerophoma with the known species of Sclerophoma before establishing a new species.

Several characteristics of this fungus are now known. It is extremely slow-growing which makes it extremely difficult to isolate. It has an optimum temperature of 20° C and grows most profusely at an initial pH of 4. Fruiting bodies are not produced on artificial media under normal laboratory conditions but develop when cultures are exposed to prolonged near-ultraviolet radiation or grown on a medium containing KNO_3 as the nitrogen source.

Much of the variation in holly scab is due to the variety of the holly that is attacked. Observations in the Astoria variety planting over the last four years would strongly support this view as well as point out the apparent lack of adequately resistant varieties. Many varieties do not react as severely as the Brownell Special

and therefore would be preferable in establishing new plantings. Greenstem varieties for example may be desirable because of their obvious tendency to produce less discoloration upon damage, even though severely effected.

It has been shown that the size of the leaf spot is also affected by the relative vigor of the host plant even though plants appear to be equally vulnerable to infection within a wide host-vigor range. In those instances where diseased plants have been kept in the greenhouse, frequently the dark red-black pigmentation usually associated with the swollen holly scab has not developed. This is probably due to lack of temperature variations which frequently are involved in anthocyanin synthesis.

There are some questions which as yet have not been answered adequately. Holly scab has been demonstrated to be a fungus-induced condition, but the organism has not been found fruiting on holly and the mechanism by which the fungus is spread under natural conditions is not known.

From inoculation trials, however, one can speculate on the nature of disease development. Under artificial conditions, distinct symptoms developed three months after inoculation (Table 8). Plants placed in the field in June showed holly scab symptoms by September (Table 8). This would indicate that holly scab inoculum is present in June, but no fruiting bodies were observed on infected

leaves.

The form of inoculum and manner of dissemination must at this time be a matter of speculation due to the absence of fruiting bodies on field infected leaves. Because of the copious production of spores in pycnidia in culture, it may be postulated that spores spread by wind and/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 and/or summer spray program seems logical. Preliminary attempts in this direction have not yielded information on scab control because of absence of the disease in the test orchard but have pointed out the hazard of chemical injury. Additional work is now under way to examine the effectiveness of different chemicals and spring and summer application in scab control.

Table 8. Time of infection and symptom development under natural and artificial conditions.

6/62	7/62	8/62	9/62	8/63	9/63
PLANTS IN GREENHOUSE-Control					
1st years new growth clean.			1st years new growth clean.	1st & 2nd years growth clean.	1st & 2nd years growth clean.
PLANTS EXPOSED TO FIELD INFECTION					
1st years new growth clean			1st years new growth spotted.	1st year spotted. 2nd year new growth clean.	1st year spotted. 2nd year new growth clean.
GREENHOUSE INOCULATIONS#1					
Inoculated			Severe spotting.		Spots on leaves until plants discarded. No spread to new growth.
GREENHOUSE INOCULATIONS #2					
Inoculated			Severe spotting		Spots on leaves until plants discarded. No spread to new growth.

In all cases of symptom development it took approximately 3 months to develop severe leaf spot symptoms.

SUMMARY

Holly scab, a leaf spot disease of English holly that is potentially a limiting factor in the production of salable cut holly, has been demonstrated to be caused by a species of Sclerophoma. This is the first report of a Sclerophoma on any member of Ilex and also the first demonstration of a Sclerophoma as a primary pathogen.

Inoculation trials involving a "Cladosporium-like" fungus earlier reported to be the cause of holly scab were negative. Cladosporium may be connected with the disease as a secondary invader or a saprophyte.

Nutritional vigor of the host does not affect the susceptibility of the host inasmuch as plants of several nutritional levels were all equally susceptible, varying only in the size of spots.

Holly scab manifests itself as irregular, translucent, or red-black swollen areas that usually are limited to the lower leaf surface. Swelling results from extreme hypertrophy and cellular proliferation of the leaf mesophyll. The color and size of holly scab spots appear to be dependent upon the variety, but observations of an infested varietal planting showed no evidence of potential resistant varieties.

Leaf abscission is not stimulated by the holly scab condition.

The holly Sclerophoma grows slowly on strep-PDA, over a range of 15° to 25° C, growing best at 20° C. It will not grow at 30° C. It grows in media having an initial pH from 4 to 7 but best at an initial pH of 4. Sporulation does not appear to be enhanced by variation in pH or temperature but is stimulated by KNO_3 as a nitrogen source or prolonged exposure to near-ultraviolet irradiation.

Other leaf spots were demonstrated to be due to physiological factors, i. e., Purple Blotch, copper toxicity, and damage associated with summer usage of nabam. The latter is being studied further.

The holly Sclerophoma is similar to S. pithyophila but, on the basis of pathogenicity on holly and morphological differences, possibly should be described as a new species. The pycnidia are non-ostiolate, spherical to pyriform and range in size from 75 microns to 200 microns. The spores are hyaline, ovoid-oblong, 2-4 x 5-7 microns with no visible sporophores at maturity but rather are embedded in a gelatinous matrix.

BIBLIOGRAPHY

1. Arruda, S. C. and C. W. Edgerton. Plants attacked by species of Spaceloma in Louisiana. (Abstract) Phytopathology 33:1. 1943.

2. Bailey, L. H. Manual of cultivated plants. New York, MacMillan, 1957. 1116 p.

3. Barnett, H. L. Illustrated genera of imperfect fungi. Minneapolis, Burgess, 1955. 218 p.

4. British Colour Council. Horticultural colour chart. London, Henry Stone and Son, 1932-42. 2 vols.

5. Brown, William M. Jr. A fungus induced leaf spot of English holly (Ilex aquifolium). (Abstract) Phytopathology 53:1138. 1963.

6. Buddenhagen, Ivan William. A Phytophthora-induced disease of English holly, Ilex aquifolium L. Master's thesis. Corvallis, Oregon State College, 1954. 66 numb. leaves.

7. Buddenhagen, Ivan William and Roy A. Young. A New Phytophthora leaf and twig blight of English holly in Oregon. Plant Disease Reporter 38:723-724. 1954.

8. _____. A leaf and twig disease of English holly caused by Phytophthora ilicis n. sp. Phytopathology 47:95-101. 1957.

9. Clements, Frederic E. and Cornelius L. Shear. The genera of fungi. New York, Hafner, 1954. 496 p.

10. Cole, J. R. Control of tar spot of holly by spraying in South Georgia. Plant Disease Reporter 35:408-410. 1951.

11. Driver, C. H. A Phyllosticta leaf-spot on Burford holly in Georgia. Plant Disease Reporter 35:412. 1951.

12. _____. Physalospora ilicis on rotundifolia holly in Georgia. Plant Disease Reporter 36:355. 1952.

13. Ducomet, Vital. Cuticularia ilicis nov. ?. Annales de l'Ecole Nationale de'agriculture, Rennes 1:235-284. 1907.

14. Ellis, J. B and F W. Anderson. New species of Montana fungi. Botanical Gazette 16:46. 1891.

15. Gomori, G. Preparation of buffers for use in enzyme studies. In: Methods in enzymology, vol. 1. New York, Academic Press, 1955. p. 138-146.

16. Grove, W. B. British stem- and leaf-fungi (Coelomycetes). Cambridge, University Press, 1935. 488 p.

17. Guba, Emil F. and John A. Stevenson. Fungus and nematode inhabitants and diseases of holly (Ilex). Amherst, 1963. 43 p. (Massachusetts Agricultural Experiment Station Bulletin 530.)

18. Herridge, Elizabeth Anne. Anatomy and pathology of leaf spots on English holly, Ilex aquifolium (Tourn.) L. Ph. D. thesis. Corvallis, Oregon State College, 1960. 89 numb. leaves.

19. _____. Pathological anatomy of leaf spots of holly. Phytopathology 53:481-487. 1963.

20. Herridge, Elizabeth Anne and R. C. Lambe. A holly leaf spot associated with the use of copper fungicides. (Abstract) Phytopathology 50:84. 1960.

21. Hawker, Lilian E. The physiology of reproduction in fungi. Cambridge, University Press, 1957. 128 p. (Cambridge Monograph in Experimental Biology no. 6)

22. Johansen, Donald A. Plant microtechnique. New York, McGraw-Hill, 1940. 523 p.

23. Leach, Charles M. Sporulation of diverse species of fungi under near-ultraviolet radiation. Canadian Journal of Botany 40:151-161. 1962.

24. Lilly, Virgil G. and Horace L. Barnett. Physiology of the fungi. New York, McGraw-Hill, 1951. 464 p.

25. Luttrell, E. S. Tar spot of American holly. Bulletin of the Torrey Botanical Club 67:692-704. 1940.

26. McWhorter, Frank P. Phomopsis canker of holly. Plant Disease Reporter 19:18. 1935.

27. _____. Scab-like spot on holly leaves in Oregon. Plant Disease Reporter 22:61. 1938.

28. Milbrath, J. A. Two unusual fungi on ornamental shrubs in the Pacific Northwest. Plant Disease Reporter 23:48. 1939.

29. Phinney, Harry K. and John R. Hardison. Immobilization of fungus spores and other minute objects in water mounts. Mycologia 46:667-669. 1954.

30. Pirone, Pascal P., Bernard O. Dodge and Harold W. Rickett. Diseases and pests of ornamental plants. New York, Ronald Press, 1960. 776 p.

31. Plakidas, A. G. Types of scab lesions on camellia. (Abstract) Phytopathology 38:21. 1948.

32. _____. Camellia scab. In: American Camellia Yearbook, 1948. Gainesville, American Camellia Society. p. 110-113.

33. _____. A convenient method for isolating slow-growing pathogenic fungi from plant tissues. Phytopathology 38:929-931. 1948.

34. _____. Corky outgrowths on camellia leaves. In: American Camellia Yearbook, 1949. Gainesville, American Camellia Society. 1. 141-146.

35. _____. Scab of Chinese holly. Phytopathology 40:22. 1950.

36. _____. Additional information on the cause and control of corky excrescenes on camellia leaves. In: American Camellia Yearbook. 1951. Gainesville, American Camellia Society. p. 204-206.

37. _____. Corky excrescence of camellia. (Abstract) Phytopathology 42:16. 1952.

38. Plakidas, A. G. Spot anthracnose of Chinese holly. Mycologia 46:346-353. 1954.

39. Roberts, A. N., R. L. Ticknor and O. C. Compton. Boron deficiency evident in Oregon holly orchards. Plant Disease Reporter 45:634-635. 1961.

40. _____. Red-leaf spotting in holly. Oregon Ornamental Nursery Digest 6(1):3. January 1962.

41. Roumequére, M. C. Fungi Gallici exsiccati. Centurie XXIII. No. 2241. Asteroma illicis Sp. nov. Grove. Révue Mycologique 5(16):214-224. 1882.

42. Saccardo, P. A. Sylloge fungorum omnium hucusque cognitorium. Pavia, Italy, the author. 1882-1931. 24 vols.

43. Shaw, Charles G. Host-Fungus index for the Pacific Northwest. I. Hosts. Pullman, 1958. 127 p. (Washington. Agricultural Experiment Station, Institute of Agricultural Sciences. Station Curcular 335.)

44. _____. Host-Fungus index for the Pacific Northwest. II. Fungi. Pullman, 1958. 237 p. (Washington. Agricultural Experiment Station. Institute of Agricultural Sciences. Station Circular 336.)

45. Simpson, Robert S. Control of leaf spot and purple blotch of American holly. American Nurseryman 115(9):50-51. May 1, 1962.

46. _____. (Holly Symposium) Missouri State Nurserymen's Annual Meeting at Columbia, Missouri, June 23-25, 1963. In: Holly Letter, Holly Society of America no. 25:8. 1965.

47. Welton, Richard Elroy. Seasonal activities of two species of eriophid mites on holly and their relation to foliage damage. Master's thesis. Corvallis, Oregon State College, 1962. 71 numb. leaves.

48. Welton, Richard Elroy and K. G. Swenson. Population cycles in eriophyid mites in holly in relation to foliage abnormalities. Corvallis, 1962. 38 p. (Oregon. Agricultural Experiment Station. Bulletin 63.)

49. U. S. Department of Agriculture. Index of plant diseases in the United States. Washington, 1960. 531 p. (Crops Research Division, Agricultural Research Service, Agriculture Handbook No. 165.)

50. U. S. Weather Bureau. Climatological data. Oregon 68-70. 1962, 1963 and 1964.

51. Wieman, John S. History of English holly (Ilex aquifolium) in Oregon and the Northwest. Portland, the author and Oregon Holly Growers Association, 1961. 39 p.

52. Young, Roy A. and Ivan W. Buddenhagen. Diseases of English hollies in the Pacific Northwest. The National Horticultural Magazine 36:108-111. 1957.