Dieback in Azaleas Caused by Phomopsis species

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Dieback, due to one cause or another, occurs in cultivated azaleas (several species and hybrids of the genus *Rhododendron* L.). A fungus belonging to the form genus *Phomopsis* Sacc. has been consistently associated with dieback in azaleas, although actual pathogenicity studies have been largely inconclusive. The primary symptoms of dieback (caused by *Phomopsis* in azaleas) are discoloration of the wood in diseased stems (Fig. 1-A) and permanent wilting and death of leaves, which usually turn reddish-brown and remain attached to the dead stems (Fig. 1-B). The objective of this study was to determine the pathogenicity of isolates of *Phomopsis* Sacc. consistently associated with and isolated from azalea cultivars. Laboratory conditions necessary to induce good sporulation of some of these isolates were studied and are reported herein. A portion of this work has been previously reported (2).

Isolations from azaleas with dieback symptoms from Charleston, S. C., consistently yielded cultures of a fungus belonging to the form genus *Phomopsis* Sacc. The association of *Phomopsis* with dieback in rhododendrons was first reported in N. J. and Pa. (4). Several other states, including Calif., S. C., and Tex., have since reported the occurrence of this fungus on either rhododendrons or azaleas (3). There was usually uncertainty in their reports as to whether *Phomopsis* caused the dieback or was merely a secondary invader. Work in Belgium (1) on a *Phomopsis* isolated from azalea revealed that conidial suspensions of the fungus infected detached leaves and stems of several plants in addition to members of the genus *Rhododendron* L. However, according to this report, inoculations made on wounded stems of azalea plants (*Azalea indicum* L.) in a greenhouse resulted in very little infection after a 3-month incubation period.

A single-spore culture of *Phomopsis* isolated from a Satsuki azalea, hereinafter called SC-A1, was used for both the in vitro and pathogenicity studies. Inoculum was prepared by culturing the fungus on carrot juice agar under constant fluorescent light at 25 C for approximately 2 weeks. Light intensity at the surface of the cultures ranged from 100 to 400 ft-c. Inoculum consisted of either 4-mm squares of sporulating mycelium on agar or a spore suspension containing approximately 1,000,000 spores/ml. Test plants were maintained either in a greenhouse where the temperature ranged from 15-35 C or in a growth chamber where the temperature was between 20 and 22 C. The azalea plants were approximately 2 years old; the rhododendrons were 6 to 12 months old. Artificial stem wounds were formed by making a shallow, longitudinal cut under the bark. Inoculated areas on stems were wrapped with moist cotton immediately subsequent to inoculation. Flowers and leaves inoculated with a spore suspension were covered with a plastic bag for 48 hr after inoculation.

Inoculations using SC-A1 were made on wounded and nonwounded stems and on nonwounded leaves of the following cultivars: Amoena, Fashion, Hino Crimson, Judge Solomon, Kaempferi, Magic Lily, Mrs. L. C. Fisher, Pink Ruffles, President Clay, and Sweetheart Supreme (azaleas); English Roseum and Jean Marie de Montague (rhododendrons). Wounded and nonwounded young leaves on azalea plants, cultivar Mrs. G. G. Gerbing, located both in the greenhouse and in the growth

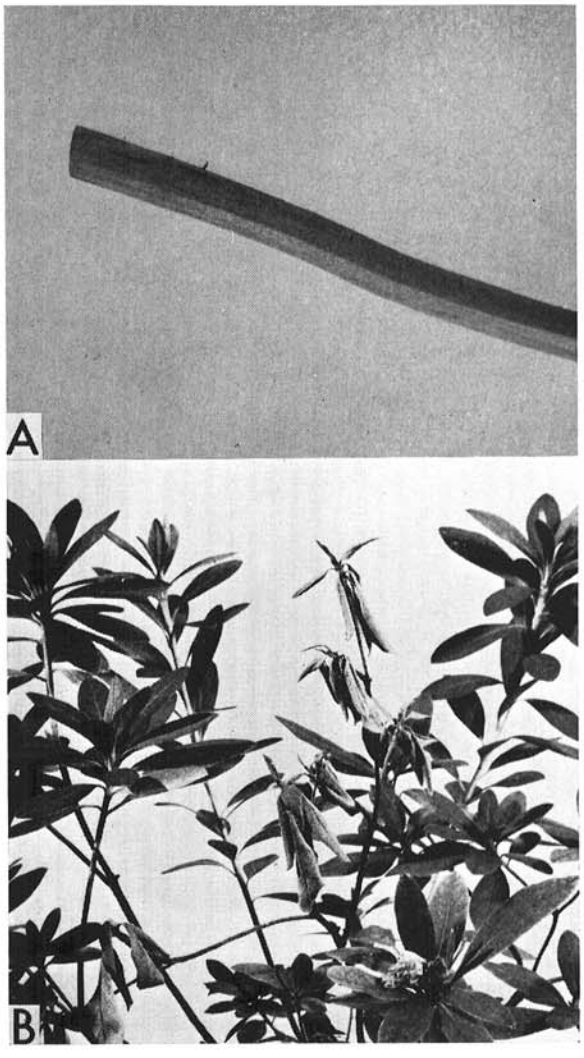

Fig. 1. Symptoms of dieback in azalea caused by *Phomopsis* sp. **A)** Discoloration of wood in diseased stem. **B)** Wilting and death of leaves.

chamber, were also inoculated. In addition, inoculations using SC-A1 were made on natural and artificially obtained leaf scars, on flowers of several azalea cultivars, and on fruits of apple, orange, and eggplant.

Studies using SC-A1 on azalea plants indicate that this fungus is chiefly a wound parasite, with stem tissue being most often affected. No infection resulted from 86 inoculations on nonwounded stems. Isolations from inoculated stems, flowers, and fruit which became infected consistently yielded pure cultures of *Phomopsis*. Isolations from controls were free of *Phomopsis*. It was not determined how long stem wounds remain susceptible to invasion, although wounds up to 8 days old served as infection courts. After a wounded stem was inoculated, the time required for dieback symptoms to appear was usually at least 2 months, but varied depending on such factors as the size and age of the inoculated stem, the variety of plant, and the ambient air temperature both at the time of inoculation and during the incubation period. Results from inoculations made on 45 leaf scars were inconclusive, but the fungus appears capable of entering stem tissue through leaf scars, since 6 of the 45 inoculated stems became infected and the pathogen was reisolated. After infection, living stem tissue is progressively killed, and eventually entire branches may be killed. Intact flowers of certain azalea cultivars were susceptible to invasion, and the fungus sporulated on several infected blossoms. Intact and wounded leaves were relatively resistant to the fungus.

The *Phomopsis* isolate also appeared to be mildly pathogenic to the stems of *Rhododendron maximum* L., *R. minus* Michaux., *Kalmia latifolia* L., *Leucothoe axillaris* (Lam.) D. Don, and *Vaccinium arboreum* Marshall.

Five isolates of the fungus from azalea varied in their virulence to the azalea cultivar Mrs. G. G. Gerbing. Three months after inoculation of wounded stems, all 24 branches inoculated with three of the isolates died, whereas only stem lesions and no death resulted from inoculation with the remaining two.

All attempts to find the perithecial stage of SC-A1 in nature or to produce it in vitro were unsuccessful. Bosmans (1) did not identify his *Phomopsis* isolate as to species, and the isolate employed in this study was not identified beyond the generic level. Positive results obtained from inoculations made with SC-A1 on apple, orange, and eggplant did not eliminate *Phomopsis mali* Roberts, *P. citri* Fawcett, or *P. vexans* (Sacc. & Syd.) Harter as being possible species responsible for azalea dieback.

The optimal temperature for radial vegetative growth of the fungus on agar media was between 25 and 28 C. Sporulation was greatly enhanced when cultures were grown in constant fluorescent light as compared with those grown in constant darkness or in alternating light and dark (13 and 11 hr, respectively). Of 10 agar media tested, including cornmeal, oatmeal, malt extract, and potato-dextrose agars, the most abundant sporulation occurred on a medium composed of Eveready carrot juice (360 ml), whole orange fruit (60 g), agar (20 g), and water (640 ml).

Phomopsis causes much of the dieback in cultivated azaleas in South Carolina. Isolates were consistently obtained from a large number of diseased azalea stems collected over the state. From 1,010 pieces of diseased stem tissue cultured, 80% of the organisms isolated belonged to the form genus *Phomopsis*. Although the disease seems to be more abundant in the coastal regions, it is widespread elsewhere over the state.

LITERATURE CITED

1. Bosmans, P. 1959. Studie van een Phomopsis sp. geisoleered vit scheutjes van *Azalea indica* L. Verh. Rijksst. Plziekt. Gent 2. 42 p.
2. Brown, S. U., & L. W. Baxter, Jr. 1969. Phomopsis dieback of azaleas. Phytopathology 59:112 (Abstr.).
3. U.S.D.A. Handbook 165. 1960. Index of plant diseases in the United States. 531 p.
4. Wood, J. I., N. E. Stevens, & P. R. Miller. 1933. Diseases of plants in the U.S. in 1932. Plant Dis. Reptr. Suppl. 85:74.