The Role of Abscised Cylindrocladium-Infected Azalea Leaves in the Epidemiology of Cylindrocladium Wilt of Azalea

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ABSTRACT

Detached azalea leaves, inoculated with either *Cylindrocladium scoparium* or *C. theae*, and placed on the rooting medium between rows of azalea cuttings in propagation flats under mist, provided inoculum which induced root and stem rot and death of many cuttings before transplanting. Less severe root and/or stem infections also occurred that did not cause wilt and death of plants until some months after transplanting. Cuttings near the inoculum source (infected leaves) died before those farther away. Of the surviving 9-mo-old azalea plants from each of three propagation flats to which *Cylindrocladium*-infected leaves were added, 36.2% (*C. scoparium* flat), and 60.6% and 28.6% (*C. theae* flats) were infected. Some of the surviving infected plants were stunted and chlorotic and showed dark streaks in the internal stem wood, and the remainder had only root infections and no above-ground symptoms.

Direct observations and indirect evidence indicated that in mist propagation flats *C. scoparium* or *C. theae* spread from infected "inoculum leaves" to healthy cuttings by: mycelial growth and splash-disseminated conidia. Conidia washed from one *C. scoparium* inoculum leaf per pot were sufficient inoculum to induce wilt on cuttings subsequently rooted therein. Furthermore, *C. scoparium* inoculum leaves placed beneath the root ball of azalea cuttings at transplanting induced root and basal stem infections, but these had not progressed to the wilt stage by 8 mo.

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Additional key words: infected carriers.

Timonin and Self (10) first described blight and wilt of azalea caused by *Cylindrocladium scoparium* Morgan. Since then, other species of *Cylindrocladium* have been reported to occur on azalea (1, 6). Of these, *C. theae* (Petch) Alfieri and Sobers (1), and *C. scoparium* were chosen for study. Both *C. scoparium* and *C. theae* may attack the aerial part of azalea plants, causing leaf spot or blight; or the below-ground parts, causing root rot, stem canker, and ultimately wilt. When temp and moisture conditions are high, both above- and below-ground symptoms may occur concurrently (4, 10). Under less humid conditions, the above-ground phase is often absent. The Cylindrocladium problem on azalea has occurred throughout the USA, and the symptoms expressed often vary according to the geographical area where the plants are grown. This variation in symptoms may be related in part to local environmental conditions, but may also be the result of transporting plants from one area to another. For example, cuttings are often propagated in one area, and then rooted cuttings or liners are shipped to other areas for further growth and use as pot plants or for landscaping. Thus, the above- and below-ground phase of the Cylindrocladium disease of azalea may become so separated by time and distance that they appear to be unrelated.

The observation that many plants soon wilt after receipt from the propagator, suggests that such plants became infected during propagation, or before or soon after shipment. The time of inoculation and the source of the inoculum, however, have not been determined. In a recent report (8), I showed that microsclerotia of *C. scoparium* or *C. floridanum* Sobers and Seymour, form in great numbers in infected azalea leaves following abscission and after the organisms have completely colonized the leaf tissue. Leaves infected with *C. theae*, although completely colonized, contained relatively few microsclerotia. Leaves which I have examined from commercial azalea propagators and/or growers, as well as those which I experimentally inoculated with either *C. scoparium* or *C. theae*, exhibited abundant conidial sporulation as well. The role such leaves might play in the disease epidemiology has not been determined, however. Bugbee and Anderson (3) showed that microsclerotia of *C. scoparium* that formed in infected spruce needles served as inoculum for root infections when mixed into the soil. Aycock (2) demonstrated that *C. scoparium* can survive at least 14 mo in infested azalea leaf tissue buried in soil. The purpose of this study was to determine what role infected azalea leaves that fall to the soil surface during or after cutting propagation, might play in the epidemiology of Cylindrocladium root rot and wilt of azaleas.

MATERIALS AND METHODS.—*Inoculation procedures.*—Cultures of *Cylindrocladium scoparium* and *C. theae* used in these studies were isolated from azalea (*Rhododendron obtusum* (Lindl.) Planch.) and maintained either on potato-dextrose agar (PDA) or V-8 juice agar (20% V-8 juice (Campbell Soup Co.), 0.3% calcium carbonate, 2% agar). Drops of conidial suspensions of each *Cylindrocladium* sp. were placed on detached azalea leaves of the cultivar 'Kingfisher', and incubated in moist chambers at 29.5 ± 1 C for 3

wk. By that time, the leaves were completely colonized and large numbers of microsclerotia had formed throughout the leaf tissue (8). Subsequently, conidial sporulation was apparent on the leaf surface. These leaves were the only source of inoculum used throughout this study, and they appeared identical to those collected and examined from commercial azalea propagators.

Ten azalea leaves infected with either *C. scoparium* or *C. theae* were used as inoculum in one experiment, and were placed centrally in a row on the surface of the propagation medium (equal parts of peat and perlite) contained in a 35 × 50 cm autoclaved flat. Kingfisher azalea cuttings were dipped in Rootone and stuck in rows parallel to the row of inoculum leaves. Each flat had 154 cuttings (11 per row, 14 rows) about 3 cm apart. Each cutting, when transplanted 8 wk later, was labeled as to its relative position in the flat. Propagation flats were placed on 24 C heat pads, and mist was controlled by an electric leaf moisture-sensing unit. Rooted cuttings were transplanted into a mixture of peat, perlite and soil (1:1:1, v/v) (which had been pasteurized with air-steam at ca. 82 C) and maintained in the greenhouse for 9 mo or until wilt symptoms appeared. Isolations from plants that wilted during this period were made by culturing surface-sterilized (0.5% NaOCl for 15 min) roots and basal-stem sections on PDA, acidified with two drops of 25% lactic acid per 25-ml plate (APDA). Isolation of *Cylindrocladium* was recorded by a number indicating the month after transplantation in which wilt symptoms appeared, marked on a grid showing the original location of the cutting in the flat. After 9 mo, the surviving plants from each flat were assayed, and isolations of *Cylindrocladium* recorded. This experiment was performed once with *C. scoparium* and twice with *C. theae*.

In another experiment, leaves on which *C. scoparium* was sporulating (inoculum leaves), were placed on the surface of greenhouse-soil mix in 10-cm diam plastic pots (one leaf per pot) placed in the mist propagation system. Control pots had no leaves placed on the soil surface. After 1 wk the leaves were removed, and newly rooted cuttings (roots 1- to 2-cm long) of azalea (cultivar 'Improved Redwing') were transplanted into the soil pots, which were left under the mist system for one more week and then transferred to the greenhouse bench. Roots and basal stem sections of plants that subsequently wilted were cultured on APDA to confirm the presence of *C. scoparium*.

In still another test, *C. scoparium*-infected inoculum leaves were chopped and placed in a mass beneath the root ball of Kingfisher rooted cuttings at the time of transplantation. Controls received no chopped leaf inoculum.

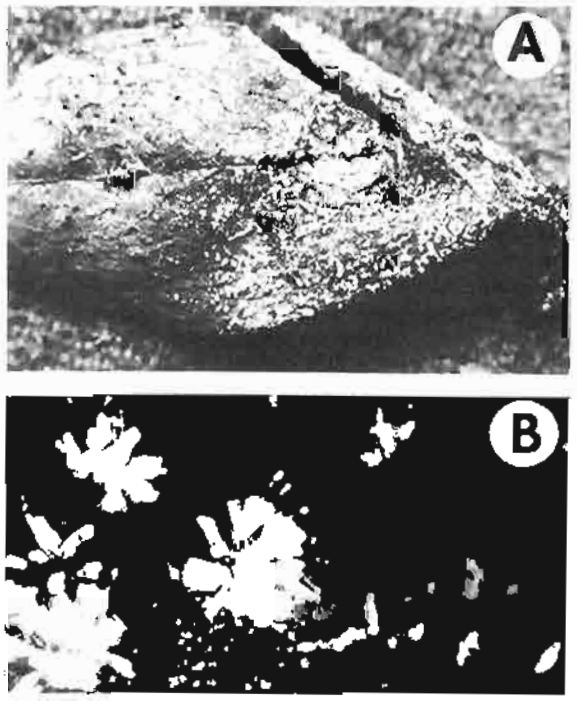

Fig. 1. Sporulation of *Cylindrocladium scoparium* on azalea (cultivar 'Kingfisher') leaves. Magnification of A = ×1 and of B = ×20. Note that conidiophores are in groups; each of the groups emerge from a microsclerotium within the leaf tissue.

RESULTS.—*Observations on leaf inoculum.*—Conidial sporulation was abundant on the surface of abscised leaves infected with either *C. scoparium* or *C. theae* (Fig. 1-A). Conidiophores of *C. scoparium* emerged directly from subepidermal microsclerotia (Fig. 1-B) (7). Conidiophores of *C. theae* developed at random on the leaf surface. They were borne primarily on surface hyphae, and were not associated with microsclerotia, which were relatively sparse or lacking. Sporulation of either *Cylindrocladium* sp. persisted on inoculated leaves for several days in a moist chamber. When conidia on such leaves were carefully washed away, new conidia were produced. Thus, conidial inoculum could be continuously supplied for prolonged periods during mist propagation.

Perithecia of the *Calonectria* perfect stage were also present on inoculum leaves infected with *C. theae* (8). The epidemiological role their ascospores may play during the mist propagation of cuttings is not known. I have examined infected leaves from commercial propagators on which perithecia of *C. theae* were present, however.

Disease development during propagation of cuttings.—In propagation flats, the first symptoms appeared on cuttings next to the row of inoculum leaves. In Kingfisher, drooping leaves, reddening of veins on the lower leaf surface (only on red- or pink-flowered cultivars), and leaf abscission were the first signs of infection. Red leaf veins and leaf abscission were caused indirectly by the production of ethylene in the infected tissue (R. G. Linderman, *unpublished*). Stem discoloration and death of wilted, defoliated cuttings followed. Mortality due to either *C. scoparium* or *C. theae* occurred throughout the

flats, but generally cuttings nearest the inoculum source died first. Mycelium of either *Cylindrocladium* sp. was observed growing from inoculum leaves into the rooting medium. In some instances, mycelium grew aerially from the inoculum leaves and infected healthy leaves. The typical leaf spot symptoms induced on the newly infected leaves verified that the mycelium observed growing from the inoculum leaves was that of *Cylindrocladium*. These newly infected leaves then abscised, and as conidiophores developed, became new sources of inoculum. New Cylindrocladium leaf spots occasionally developed on leaves of cuttings remote from the original inoculum leaves; that is, nearer the ends of the flats. This indicated either that spores had been splashed from infected leaves in the mist system and infected a healthy leaf, or aerial mycelium had somehow induced a single lesion. If that infected leaf abscised, it too became a source of inoculum, although leaves with single-spot infections often did not abscise, or did not abscise until several months after being transplanted.

Direct observations indicated that conidia were washed or splashed from abscised, infected leaves, and therefore could directly infect new roots of cuttings. In addition, the efficacy of conidia as inoculum washing into the rooting medium before roots were present was also demonstrated. Ten rooted cuttings were stuck into greenhouse soil-mix on which one *C. scoparium*-infected inoculum leaf had been placed for 1 wk. Two rooted cuttings developed wilt symptoms within 5 wk, and six of ten wilted and died within 8 wk. None of the control plants wilted, and when cultured, none was infected with *C. scoparium*. This experiment demonstrated that conidia that washed into the soil had either persisted, as such, or germinated and given rise to mycelium that colonized the medium. Either way, root infections were initiated which ultimately caused wilt and death.

Disease development after transplanting cuttings.—Experiments in propagation flats were terminated 9 mo after the rooted cuttings were transplanted. The basal stem and roots of all surviving plants were cultured on APDA, and the distribution of plants carrying *C. scoparium* or *C. theae* was noted (Fig. 2). The distribution and time when wilt symptoms appeared on plants that died during the 9 mo are also depicted in the grids (Fig. 2). Data relating to these experiments are tabulated in Table 1. In general, *C. scoparium* and *C. theae* were equally aggressive and pathogenic to cuttings during propagation. The majority of the plants that wilted and died did so at or within the first month after transplanting, and within two to three rows of the original inoculum source, although there were some obvious exceptions. Although not wilted, some surviving plants that were shown to have *Cylindrocladium* infections in their roots or stems, were stunted and had shown obvious nutrient-deficiency symptoms (chlorosis) for several months. In addition, the internal wood was frequently discolored several centimeters up the stem.

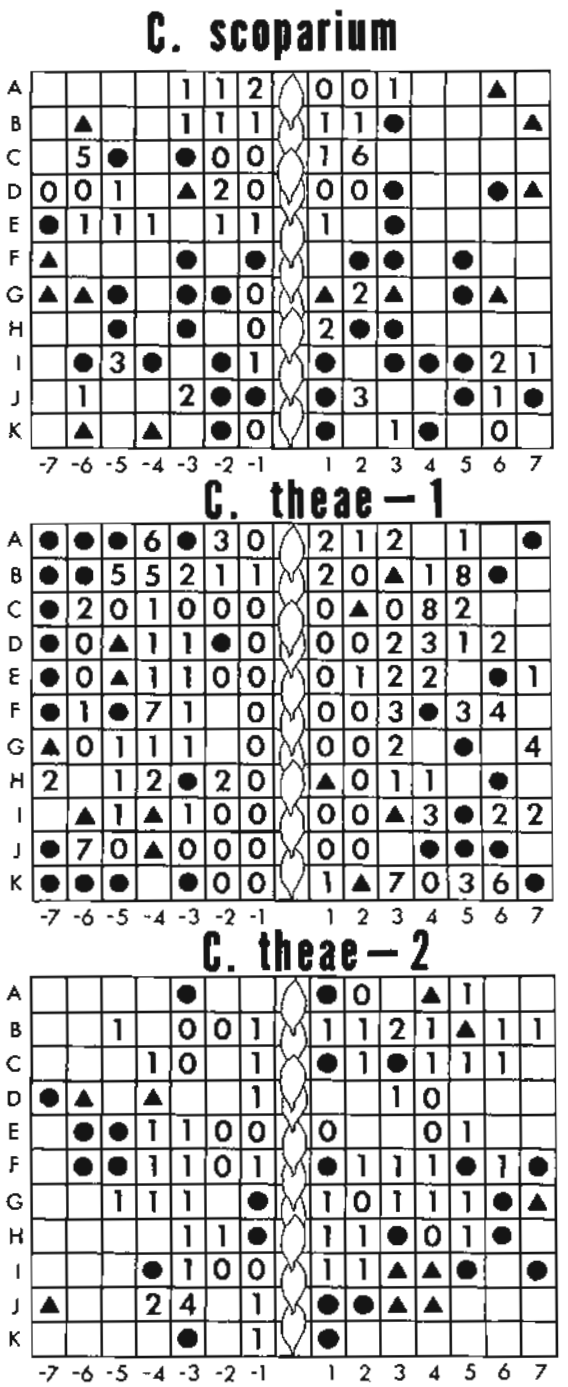

Fig. 2. Grids showing infection of azalea cuttings (cultivar 'Kingfisher') propagated in the presence of a row of 10 azalea leaves infected with either *Cylindrocladium scoparium* or *C. theae*. Each grid space indicates the position of each cutting in the flat relative to the inoculum. Numbers in the grid spaces indicate the month after cutting transplantation in which the plant wilted and died from *Cylindrocladium* infection; solid triangles indicate mortality within 9 mo without isolation of *Cylindrocladium*; solid circles indicate plants which survived for 9 mo, but which carried *Cylindrocladium* in their roots or stem. Blank spaces indicate healthy plants free of *Cylindrocladium*.

TABLE 1. Infection by, and reisolation of two pathogenic *Cylindrocladium* spp. from, azalea cuttings (cultivar 'Kingfisher') rooted in flats inoculated with a row of azalea leaves infected with either *C. scoparium* or *C. theae* (see Fig. 2)

Mortality at 9 mo: Pathogen reisolation	% host survival and pathogen reisolation from cuttings originally inoculated with:		
	C. scoparium	*C. theae*	
		Exp. 1	Exp. 2
Died within 9 mo:			
Cylindrocladium recovered	28.6[a]	61.7	39.0
Cylindrocladium not recovered	8.5	7.2	6.5
Survived 9 mo:			
Cylindrocladium recovered	22.7	18.8	15.5
Cylindrocladium not recovered	40.2	12.3	39.0
Total survivors	62.9	30.1	54.5
Survivors infected with *Cylindrocladium*	36.2[b]	60.6[b]	28.6[b]

[a]% of flat containing 154 azalea cuttings.

[b]% of total survivors.

The remainder carried *Cylindrocladium* in their roots or stem but showed no above-ground symptoms. The percentages of infected plants surviving 9 mo after transplanting from the *Cylindrocladium*-infested flats (one flat with *C. scoparium*, and two with *C. theae*) were 36.2, 60.6, and 28.6%, respectively (Table 1).

In an additional experiment, chopped leaves infected with *C. scoparium*, which were placed beneath six rooted cuttings as they were transplanted, had not induced wilt within 8 mo. However, *C. scoparium* was recovered from the basal portion of stems of five of the six plants, indicating that root and stem infection had occurred. *Cylindrocladium* was not recovered from any of the control plants.

DISCUSSION.—The intent of this study was to determine what role *Cylindrocladium*-infected azalea leaves, which abscise during propagation, may play in the epidemiology of the root rot and wilt phases of the disease on azalea. The study demonstrated that abscised, infected leaves are an extremely effective source of inoculum in azalea cutting propagation beds. They produce abundant conidia and mycelial growth, which infest the rooting medium and infect stems, leaves, and new roots of cuttings. In addition, conidia apparently may splash from the original inoculum leaf and cause new leaf spot infections which, if the infected leaf abscises, can also establish an infection center.

This study further demonstrated, as did that of Bugbee and Anderson (3) on spruce, that infected, abscised leaves with or without microsclerotia do link the above- and below-ground phases of the Cylindrocladium disease on azalea. That is, some of the root infections that result in a wilted plant in one geographical area can probably be traced back to a *Cylindrocladium*-infected leaf that abscised in the cutting bed. The inoculum from that leaf may have killed some cuttings immediately; in others it produced less severe root infections requiring more time to induce wilt symptoms. Detection of an occasional abscised, infected leaf in a propagation bed may be very difficult, however, since cuttings are placed so close together that the infected leaf would probably be hidden. Such infections would not be detected unless an obvious infection center became apparent. Plants in such a center should be removed, but it is essential that peripheral plants be discarded too, since they are likely to be symptomless carriers of the pathogen. If not discarded, such plants would then be shipped to some other location where wilt symptoms would eventually be expressed. In this regard, this study has experimentally duplicated and partially explained what has already been observed in the azalea industry.

Leaves with Cylindrocladium leaf spot may also play a role in the disease epidemiology if they abscise after the rooted cutting is transplanted from propagation flats into the field or container. If such infected leaves abscise and remain on the soil surface, they could produce conidia that wash down to the roots of the plant. Root infections that result may not produce wilt symptoms for months, possibly years. The variable length of that period may depend largely on growing conditions. If growth is rapid and new root formation is vigorous, the period might be quite long. Poor growing conditions or certain cultural practices may enhance disease development and thereby shorten the period until wilt symptoms appear. It was also demonstrated here that infected leaves mixed into the soil can induce root and stem infections, even though in this study the infected plants had not wilted in 8 mo. The report by Aycock (2) that infested azalea leaves can survive at least 14 mo in soil serves to further emphasize the significance of infected leaves in the epidemiology of the Cylindrocladium wilt disease of azalea. His report also draws attention to apparent superficial stem lesions on mist-rooted cuttings which may also give rise to eventual wilt. His work, and that reported here, emphasize the need for sanitary cultural practices in addition to effective fungicide spray programs on stock plants and cutting dips to eradicate both stem and leaf infections (2, 5, 9).

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