Ascospore Discharge from Perithecia of Calonectria theae, C. crotalariae, and C. kyotensis

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ABSTRACT

Ascospores were discharged from perithecia of *Calonectria theae, C. crotalariae*, and *C. kyotensis* on agar cultures, or on detached infected azalea leaves. Ascospore discharge from perithecia on leaves was greater at relative humidities (RH's) of 93% or less, than at 100% RH. Discharged ascospores from perithecia on infected leaves were viable. Oozed ascospores of *C. theae* infected healthy detached azalea leaves as readily as conidia.

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Additional key words: *Cylindrocladium* spp., peanut, *Acacia* spp., papaya, *Rhododendron*.

Perithecia of several species of *Calonectria* have been observed on host tissue in nature, or under experimental conditions. Of interest to me were those species having a *Cylindrocladium* imperfect stage, namely *Calonectria theae* Loos, *C. crotalariae* (Loos) Bell & Sobers, and *C. kyotensis* Terashita (=*C. floridana* Sobers, and *C. uniseptata* Gerlach). *Calonectria theae* is a pathogen on azalea (*Rhododendron obtusum* (Lindl.) Planch) (1), and its perithecia were shown to occur on abscised, artificially infected azalea leaves (8), and the author has observed them on naturally infected, abscised leaves from commercial azalea cutting propagation beds. *Calonectria crotalariae* is a serious pathogen on peanut (*Arachis hypogaea* L.) (4), *Acacia koa* A. Gray (2), and papaya (*Carica papaya* L.) (7, 9). Its perithecia were observed at the soil line on the dead stems of all three hosts. *Calonectria kyotensis* occurs on peaches (*Prunus persica* (L.) Batsch) (10, 11), *Acacia* spp. (12), and redbud (*Cercis* spp.) (5), and two of these reports (10, 12) indicated that perithecia occur on infected host tissue under experimental conditions. Perithecia of certain homothallic isolates of all three of these *Calonectria* species, however, are readily produced on V-8 juice agar and on inoculated detached azalea leaves.

It has generally been observed that ascospores of *Calonectria* spp. ooze in masses from perithecia produced in culture or on other substrates under high-moisture conditions (12). In nature, these masses of ascospores would presumably be dispersed by splashing water. The increased incidence and rapid spread of *Cylindrocladium* diseases where perithecia of the *Calonectria* stage are produced (3), suggests the possibility of an airborne phase in which inoculum may be dispersed in air currents. The purpose of this study was to determine whether ascospores can be forcibly discharged from perithecia produced on agar or on infected azalea leaves, and whether ascospores can infect azalea leaves.

MATERIALS AND METHODS.—*Production of perithecia.*—Detached azalea leaves of cultivar 'Kingfisher' were separately inoculated with *Cylindrocladium* conidia of each isolate of each *Calonectria* sp. tested, and maintained in a moist chamber. Perithecia formed after the leaf was totally necrotic, usually 2-3 wk after inoculation (8). The leaves bearing perithecia were then transferred to 15 × 60 mm plastic petri-dish bottoms covered with a matching inverted petri-dish bottom. The inverted dish contained 10 ml of agar, to which discharged ascospores could adhere and establish a colony. A gap of 1-3 mm separated the edges of the two dishes. The agar medium was potato-dextrose agar, supplemented with 1 gm 0.75% pentachloronitrobenzene (PCNB) per liter, and 1 drop of 25% lactic acid/10 ml medium. Because colonies developed slowly in this medium, the number of discharged ascospores could be estimated.

Perithecia were also produced on 2- to 3-wk-old V-8 juice agar cultures (10% V-8 juice, 0.3% $CaCO_3$, 2% agar/liter). These cultures were covered with an agar-trap in the same manner as the leaves bearing perithecia.

Perithecia, produced on azalea leaves or on agar cultures and covered with an agar-trap, were incubated in 500-cc jars containing ca. 200 cc perlite and 100 ml of various glycerol solutions (Fig. 1). These solutions were prepared in various ratios with water to establish a range of relative humidities (RH) from 16.9 to 100% (6). The jars were generally kept on the lab bench (ca. 25 C).

The isolates used in this study all produced abundant perithecia: *Calonectria theae* isolated from azalea; *C. kyotensis* isolated from redbud; and *C. crotalariae* isolated from peanut or *Acacia koa*.

Infectivity of ascospores was determined by collecting ascospore ooze from perithecia maintained at a high RH, suspending them in water, and adding droplets of the suspension to healthy detached azalea leaves.

RESULTS.—Perithecia of all test isolates of *Calonectria theae*, *C. crotalariae*, or *C. kyotensis* discharged ascospores either from V-8 juice cultures or from infected azalea leaves. These ascospores all germinated and established small colonies on the agar trap plates. Numerous attempts were made to determine the optimum RH for ascospore discharge. The general conclusion from these tests, some of which involved RH's from 16.9% to 100%, was that ascospores were discharged from perithecia on leaves at all RH levels tested, but generally more were discharged at RH <100%. In one experiment with *C. theae*, more ascospores were discharged from perithecia on leaves at 44 and 85% RH than at 100% (Table 1). The perithecia on leaves held at 44 and 85% RH did not ooze ascospores, and within 48 h appeared dried and somewhat shrivelled, as did the leaves themselves. In contrast, perithecia on leaves at 100% RH remained moist, and ascospore ooze from most of the perithecia was apparent.

Ascospore ooze was collected from perithecia of *C. theae* and suspended in water. Droplets of this suspension were placed on detached Kingfisher azalea leaves. Comparable concns of conidia were added in a parallel series as a standard for infectivity. Ascospores and conidia were equally infective at all the dilutions tested.

DISCUSSION.—Perithecia of *Calonectria* spp. generally ooze ascospores in a slimy mass when moisture conditions are high, but the present study has

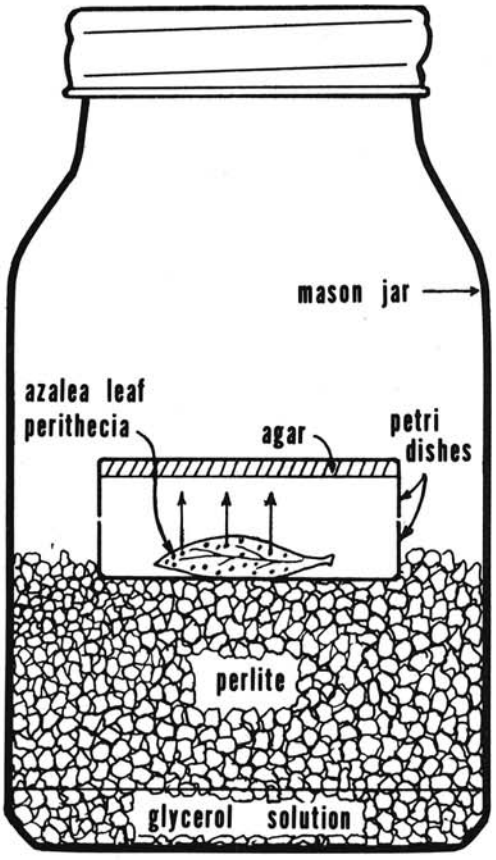

Fig. 1. Diagram of Mason-jar relative-humidity (RH) chamber used to control RH for ascospore discharge from perithecia of *Calonectria* spp. on infected azalea leaves. Glycerol solutions in the bottom were used to establish specific atmospheric relative humidities (6).

TABLE 1. Estimated numbers of ascospores discharged and trapped on agar plates inverted over infected leaves with perithecia of *Calonectria theae* placed at three relative humidities (RH). Two readings were taken on two replicate leaves 2 and 7 days after transfer to different RH.

Relative humidity	Time interval	Number of discharged ascospores A	B	Avg(A+B)/2
100%	0-2 days	0	100	
	5-7 days	150	0	
		150	100	125
85%	0-2 days	59	500	
	5-7 days	0	0	
		59	500	280
44%	0-2 days	500	500	
	5-7 days	0	0	
		500	500	500

demonstrated that they may also be forcibly discharged into the air. More ascospore discharge appears to occur at RH <100%, or as a result of a change from a higher to a lower RH. High RH, and probably even free water, is necessary for perithecium formation and maturation. If these conditions persisted, ascospores would ooze from most of the perithecia. However, a slight decrease in RH when the perithecia are mature apparently induces some physiological changes that cause the ascospores to be forcibly discharged into the air, and in this study the distance was at least 2 cm. Since ascospores are viable and infective, there is no reason why they should not be considered potentially significant inoculum for infection on susceptible hosts, especially in areas of high humidity or high rainfall (9).

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