Natural Occurrence of *Microsphaera pulchra* and *Phyllactinia guttata* on Two *Cornus* Species

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ABSTRACT

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Powdery mildew has become a common foliar disease of *Cornus florida* and other dogwood species in the eastern United States during the last several years. This study was conducted to determine the identity of powdery mildew fungi on *C. florida* and *C. amomum*. Ascocarps of *Microsphaera pulchra* and *Phyllactinia guttata* occurred singly and together on both *C. florida* and *C. amomum* leaves. *M. pulchra* ascocarps occurred at a higher density than *P. guttata* ascocarps on *C. florida* leaves, whereas *P. guttata* ascocarps occurred more frequently than *M. pulchra* ascocarps on *C. amomum* leaves. Histological studies, however, did not provide supplementary data of infection by the powdery mildew species that occurred less frequently on the leaves of each dogwood species. *M. pulchra* did not penetrate the cells of *C. amomum*, and likewise *P. guttata* did not enter through stomata of *C. florida* leaves. The presence of ascocarps of both species was not the result of infection of the dogwoods by both pathogens. The ascocarps of *M. pulchra* probably became airborne and then settled on the *C. amomum* leaves. Similarly, the ascocarps of *P. guttata* landed on *C. florida* leaves. These results emphasize the importance of correct pathogen identification using several criteria such as ascocarp morphology, host-pathogen relationships, distribution of the pathogen, conidial morphology, and histology.

Additional keywords: *Oidium*, *Ovulariopsis*, scanning electron microscopy

There are approximately 65 *Cornus* L. species (dogwoods; 4) distributed throughout the northern hemisphere, with centers of diversity in eastern North America, the Pacific Northwest, eastern Asia, and Central America (10). Flowering dogwood, *C. florida* L., is an understory species native to the deciduous forest in the eastern United States and plays an important role in the ecosystem by providing habitat and food for wildlife (4). Several species of dogwoods are cultivated for their showy bracts, attractive foliage, and fruit and twig color.

Powdery mildews of *Cornus* spp. were rarely reported before 1994, but have since become a common foliar disease in the eastern United States (6,9,11,12). Dogwood leaves infected with powdery mildew become distorted, have an increase in red pigmentation, and develop lesions that eventually become necrotic. The disease may also cause stunting of young seedlings and slow growth in older trees (12). Nearly 100% of the foliage may be affected in

seedlings and liners, resulting in possible mortality, whereas older, well-established trees generally are not killed by the disease.

Two powdery mildew species have been reported to infect dogwoods in eastern North America, *Microsphaera pulchra* Cooke and Peck (1,5,11) and *Phyllactinia guttata* (Wallr.:Fr.) Lév. (2,5,9). The anamorph of *Microsphaera* spp. (*Oidium*) was reported on flowering dogwood (5,6), whereas *Ovulariopsis*, the anamorph stage of *Phyllactinia* spp., was reported on various *Cornus* spp. (1). *P. guttata* is the only species within this genus reported to occur on *Cornus* spp. (1,5).

C. florida is valued for its pink or white bracts, red fruit, and seasonal red foliage (13). *C. amomum* Mill., the silky dogwood, is a common blue-fruited shrub found in swampy areas of the eastern United States (4). The purpose of this study was to identify the powdery mildew species infecting *C. florida* and *C. amomum* trees that were grown in a research compound on The University of Tennessee Agricultural Campus.

MATERIALS AND METHODS

Leaf disks were collected from three *C. florida* trees and one *C. amomum* tree with powdery mildew in the fall of 1996 on The University of Tennessee Agricultural Campus. The dogwood trees were located at

opposite corners (11 m apart) of a research tree compound. Disks were cut from the leaves using a 7-mm-diameter cork borer, bisected, then fixed and vacuum aspirated in Histochoice (Amresco, Solon, OH). Leaf samples were then dehydrated with a series of 30, 50, 70, 90, 95, and 100% isopropyl alcohol each for 30 min. Dehydrated samples were embedded with TissuePrep (Fisher Scientific Co., Fair Lawn, NJ) and cast into blocks. Leaf disks were sectioned at 10 µm on a rotary microtome and placed on glass slides. Sections were stained with a modified Flemming's Triple Stain (8). Cover slips were applied with Eukitt Mounting Medium (Calibrated Instruments, Hawthorne, NY) and slides viewed with a Nikon Diaphot compound microscope (Nikon Corp., Tokyo) at 400 to 1,000× magnification. Photographs were taken with a Nikon N2000 camera using Techpan 2415 film (Eastman Kodak Company, Rochester, NY).

Additional leaves collected from the same *C. amomum* and *C. florida* trees were dried in a plant press. A separate species of powdery mildew was observed on the leaves of each dogwood species using an Olympus SZH10 research stereo dissecting scope (Olympus America Inc., Lake Success, NY) at 40 to 70× magnification. *P. guttata* was identified on *C. amomum* using number of asci/fruitlet body, ascocarp diameter, and form of appendages (1). The powdery mildew observed on *C. florida*, a *Microsphaera* sp., was identified using asci numbers/ascocarp, form and length of appendages, and ascocarp size. Eight *Microsphaera* spp. ascocarps, from a total of 3 dried leaf specimens of *C. florida*, were mounted in lactophenol and examined with an Olympus BH-2 compound microscope at 100 to 400× magnification. The diameter of these 8 ascocarps and the length of 4 to 5 appendages of each ascocarp were measured. The final ratio of appendage length to ascocarp diameter determined the identity of the *Microsphaera* sp. (1).

Using a 12-mm cork borer, disks were cut from dried leaves of each dogwood species that had both *M. pulchra* and *P. guttata* ascocarps occurring together, and were prepared for scanning electron microscopy without fixation or dehydration. Specimens were mounted on aluminum stubs with double-sided sticky tape, sputter-coated with gold-palladium, then viewed with an ETEC Autoscan (Hayward,

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CA) at 60 to 1,200× magnification with an operation at 10 kV. Photographs were taken on panchromatic Polaroid 4 by 5 instant sheet film (Polaroid Resource Center, Cambridge, MA).

RESULTS

Dichotomously branched appendages and the presence of many asci in each ascocarp identified one of the powdery mil-

dew genera as *Microsphaera* (1). Species determination was based on the ratio of ascocarp diameters to appendage length. The ratio of ascocarp diameter (92-(107)-128) to appendage length (117-(134)-160) was $1:1.3 \pm 0.14$, which identified the species as *M. pulchra* (1). Bulbous-based appendages and number of asci/ascocarp identified the second powdery mildew species as *P. guttata* (1).

Ascocarps of *P. guttata* and *M. pulchra* were observed on some dogwood leaves (Fig. 1). *M. pulchra* ascocarps were distributed randomly on the abaxial surface of *C. florida* leaves and occurred at higher densities than ascocarps of *P. guttata*. *P. guttata* ascocarps were mixed with and in close association with *M. pulchra* ascocarps on these same leaves. In contrast, ascocarps of *P. guttata* were commonly found on *C. amomum* leaves in close association with the less frequently encountered *M. pulchra* ascocarps. Ascocarps of *M. pulchra* on *C. amomum* were at times directly on the leaf surface and at other times appeared attached to the *P. guttata* ascocarps. However, *P. guttata* ascocarps often were situated on the *C. florida* leaf surface with appendages pointing away from the epidermis.

Histological studies revealed that *P. guttata* hyphae entered the abaxial leaf surface of *C. amomum* through stomatal openings (Fig. 2A). The hyphae ramified throughout the leaf tissue of the host and produced haustoria only in mesophyll cells. *M. pulchra* hyphae were restricted to the surface of the *C. florida* leaf and penetrated directly, forming haustoria only in the adaxial and abaxial epidermal cells (Fig. 2B). Since penetration through stomata was not observed on *C. florida* leaves, it was assumed that *P. guttata* was not a pathogen of these leaves. Likewise, since direct penetration was not observed on *C. amomum* leaves, it was assumed that *M. pulchra* did not infect these leaves.

DISCUSSION

Yarwood (14) cautioned that errors of host-parasite association can be made if ascocarps of powdery mildew fungi become associated with a plant after dissemination. Appendage orientation of *Phyllactinia* spp. was used to determine the origin of ascocarps on specific leaf surfaces (3).

Fig. 1. Powdery mildew on *Cornus amomum*. Occurrence of *Microsphaera pulchra* (MA) and *Phyllactinia guttata* ascocarps (PA) on the abaxial leaf surface. P = appendages of *P. guttata*; M = appendages of *M. pulchra*; bar = 55 μ m.

Fig. 2. (A) Penetration of *Cornus amomum* abaxial epidermal cells by *Phyllactinia guttata*. G = guard cells; P = *P. guttata* hyphae; bar = 12 μ m. (B) Penetration of *C. florida* adaxial epidermal cell (E) by *Microsphaera pulchra*. M = *M. pulchra* hyphae; H = Haustoria; bar = 13 μ m.

Phyllactinia spp. have the following two appendage types: bulbous equatorial appendages and branched appendages formed on the crown or apex of the fruiting structure. The bulbous appendages point downward and function to free the mature ascocarp from superficial mycelium on the leaf surface (3). The ascocarp adheres to new surfaces with the apical appendages downward and the bulbous-based appendages arranged so that the tips point away from the surface (3). *P. guttata* ascocarps on the *C. florida* leaves had bulbous-based appendages pointing away from the leaf surface, which implied the structures were not formed on these dogwoods. *M. pulchra* appendages appear not to function to lift the ascocarp from host surface. Therefore, histological examination of the infected tissue for the mode of pathogen ingress and colonization of the leaf would be needed to determine if the ascocarp had formed on the leaf.

Histological studies were conducted to determine ingress and colonization by powdery mildew pathogens of each host. The mycelium of *Microsphaera* spp. is epiphytic, whereas the mycelium of *Phyllactinia* spp. is hemiendophytic, forming both mycelium on the surface and producing internal hyphae (1,14). The mycelium on the abaxial leaf surface of both dogwood species was profuse, and ascocarps were readily found in the histological sections of the leaves. Hyphae of *P. guttata* entered stomata of the *C. amomum* leaves; however, there was no indication that *P. guttata* hyphae entered the stomata or grew within mesophyll tissue of *C. florida* leaves. *M. pulchra* directly penetrated the abaxial and adaxial epidermal cells of *C.*

florida, but did not infect *C. amomum*. This study provides documentation of both *M. pulchra* and *P. guttata* ascocarps simultaneously occurring on *C. florida* and on *C. amomum*; however, there was no evidence of concurrent infection by both fungi within the same dogwood leaves.

Powdery mildews can be identified using five interrelated criteria. The most conclusive way to identify the pathogen is with ascocarp morphology. However, ascocarps are not formed until late in the fall, if at all, on dogwoods. Previously documented reports of host-pathogen relationships and distribution of the pathogen are useful supplements to ascocarp morphology (7). Throughout the growing season, the conidial stage can be used to identify the pathogen, but environmental conditions may cause variations in the morphology (1). The most accurate technique of identification is histological examination of host tissue that reveals penetration of the leaf cell, morphology and area of haustorial formation, and the area of hypha ramification.

Observations of ascocarps of two powdery mildew species occurring on a dogwood species warrants caution. Several criteria need to be utilized to identify the powdery mildew pathogen of a particular host. Potential differences in the disease processes of *Microsphaera* and *Phyllactinia* spp. may have implications to breeding programs in the search for a general resistance mechanism for disease management. Yarwood warned that resistance to one powdery mildew species may not necessarily correlate with resistance to another (14); therefore, correct identification of the pathogen is imperative.

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