Spore Germination, Infection Structure Formation, and Colony Development of *Erysiphe pulchra* on Dogwood Leaves and Glass Slides

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ABSTRACT

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Spore germination, infection structure formation, and colony development of *Erysiphe pulchra* on glass slides and leaf disks of a susceptible flowering dogwood line were examined using light and scanning electron microscopes. On both glass slides and leaf disks, germination of conidia started within 2 h after inoculation (hai). One to four germ tubes grew from two poles of a conidium, one or two of the germ tubes formed initial appressoria, and only one of the germ tubes with initial appressoria formed secondary appressoria. However, formation of initial and secondary appressoria was delayed on glass slides (48 and 72 hai, respectively) compared with that on dogwood leaf disks (3 and 24 hai, respectively). Branching hyphae did not grow from germinated conidia on glass slides. However, on dogwood leaf disks, branched hyphae were observed 48 hai. In epidermal cells, the fungus formed compact and globose haustoria. Conidia formation on conidiophores started on leaf disks 7 days after inoculation.

Additional keywords: *Cornus florida*, infection process, *Microsphaera pulchra*, powdery mildew

Flowering dogwood (*Cornus florida* L.) is used as an important ornamental tree in North America, Japan, and Europe. Since 1995, epidemics of powdery mildew caused by *Erysiphe pulchra* (Cooke and Peck) U. Braun & S. Takamatsu (syn. *Microsphaera pulchra* Cooke and Peck) have impeded production of flowering dogwood throughout much of the eastern United States. The fungus is an obligate parasite and grows mainly on the leaf surface. The disease can cause stunted, distorted growth or twig or plant death, which can be undesirable aesthetically. Using resistant cultivars is a major strategy in the management of powdery mildew on flowering dogwood in landscapes, and several resistant cultivars have been released (15,16).

Conidia of most fungi causing powdery mildew start to germinate on leaf surfaces within 2 h after inoculation (hai; 4,11,13,14). However, patterns of infection structure formation differ in some pathosystems of powdery mildew. For most of the powdery mildew fungi, such as *E. pisi* DC. on sweet pea (*Lathyrus odoratus* L.; 13) and pea (*Pisum sativum* L.; 14), *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci on melon (*Cucumis milo*; 12), and *Oidium lycopersicum* Cooke & Massee (Noordeloos & Loerakker 1989) on *Lycopersicon* spp. (10), a conidium germinates and produces the primary germ tube ending in a primary appressorium, and hyphae arise from the primary appressoria and the body of conidia. In contrast, for *Blumeria graminis* (DC.) E. O. Speer (syn. *E. graminis* DC. f. sp. *hordei* Ém. Marchal) on barley, the initial germ tubes do not form appressoria, but the secondary germ tubes form hook-shaped appressoria and hyphae develop from appressoria (1,8,11).

Considered a new disease in the United States, little is known about the infection process of *E. pulchra* on dogwood. Understanding early stages of pathogen development on the host plant is essential to any future investigation of mechanisms of resistance to powdery mildew. The objective of the present study was to describe the infection process, including conidial germination, infection structure formation, and colony development, of *E. pulchra* on flowering dogwood.

MATERIALS AND METHODS

Inoculum source. Inoculum of *E. pulchra* was maintained throughout the year on flowering dogwood 'Cherokee Princess' trees in a greenhouse at the University of Tennessee, Knoxville. Trees were shaken to remove old conidia from colonies 2 days before using diseased leaves as an inoculum source.

Plant material. Three-year-old susceptible flowering dogwood trees, MW 94-60 (a wild seedling selected for high susceptibility), were maintained in a greenhouse at the University of Tennessee. On growing dogwood shoots, fully expanded leaves were collected and washed with running distilled water for 1 min. Leaf disks were cut using a 1.5-cm-diameter cork borer. The experiment was conducted during April to July in 2004.

Inoculation. Leaf disks (adaxial surface up) and glass slides were laid on two layers of moistened filter paper in petri dishes. Leaf disks in petri dishes were placed at the bottom of a 0.44-m high, 0.26-m-diameter settling tower and inoculated with conidia from several powdery mildew-infected leaves. Within the settling tower, conidia were released into the air using a blast of air over infected leaves hung at the top of the tower. The air blast was repeated six to eight times. Conidia were allowed to settle on the leaf disks for 2 min before assessing conidial deposition microscopically. Inoculum density was monitored by counting the number of conidia per square centimeter on a glass slide

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Table 1. Time of conidium germination, appressorium formation, and branched hyphal growth of *Erysiphe pulchra* on glass slides and flowering dogwood leaf disks

	Time (h)			
Substratum	Conidium germination	Initial appressorium formation	Secondary appressorium formation	Branched hyphal growth
Leaf disk	2	3	24	48
Glass slide	2	48	72	…[a]

[a] No branched hyphal growth even 7 days after inoculation.

placed in the settling tower. The spore density on leaf disks and slides was adjusted to 400 to 600 conidia/cm^2 by repeated blasts of air. Inoculated leaf disks and slides were incubated at 20ºC with a 19-h photoperiod; water was added to the filter papers as needed to maintain high relative humidity.

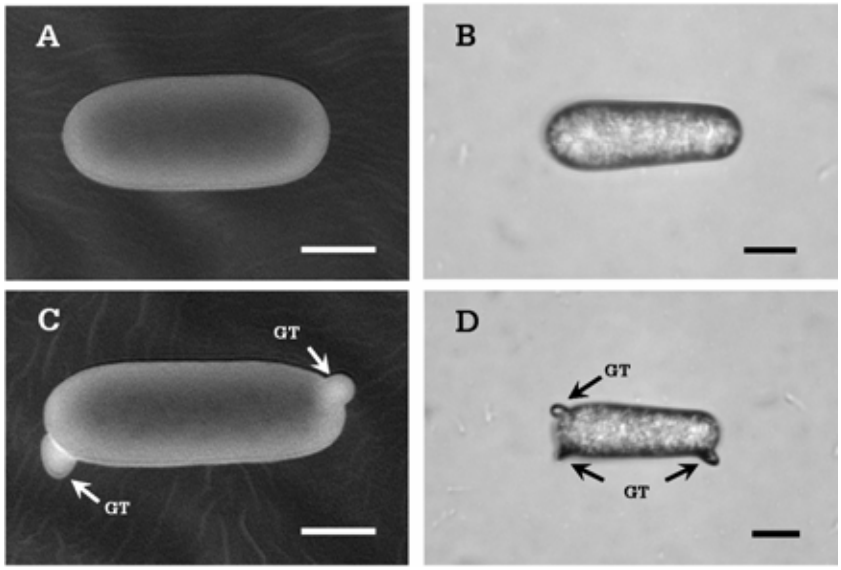

Fig. 1. A and **C**, Scanning electron micrographs and **B** and **D,** light micrographs of spore germination of *Erysiphe pulchra*. **A,** Conidium on the leaf surface 1 h after inoculation (hai). **B,** Conidium on a glass slide 1 hai. **C,** Germinated conidium with germ tubes (GTs) on leaf surface 2 hai. **D,** Germinated conidium with GTs on a slide 2 hai. Bars = 10 µm.

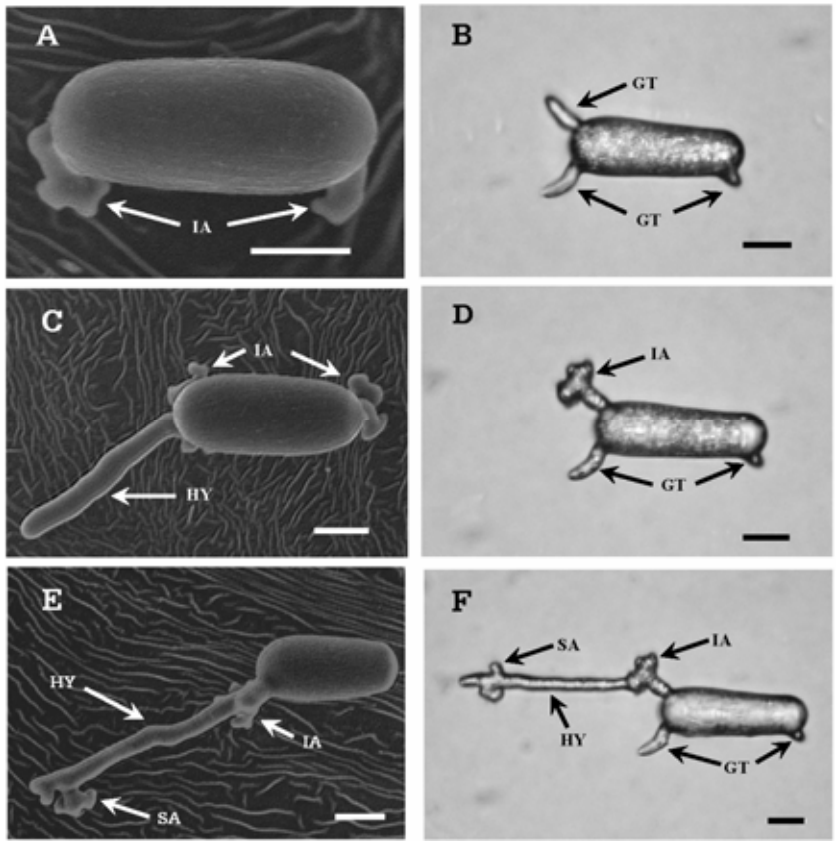

Fig. 2. A, **C,** and **E**, Scanning electron micrographs and **B, D,** and **F,** light micrographs of infection structure formation of *Erysiphe pulchra* on dogwood leaves and glass slides. **A,** Initial appressoria (IA) from germ tubes (GTs) at both poles of a conidium on the leaf surface 3 h after inoculation (hai). **B,** Continuous growth of GTs without appressorium formation on a glass slide 12 hai. **C,** Hypha (HY) growth from the initial appressorium on leaf surface 12 hai. **D,** Initial appressorium on a glass slide 48 hai. **E,** Germinated conidium with secondary appressorium on leaf surface 24 hai. **F,** Secondary appressorium (SA) formation on a slide 3 days after inoculation. Bars = 10 µm.

Scanning electron microscope examination. Inoculated leaf disks sampled from the petri dishes at 1, 2, 3, 6, 12, and 24 hai and 2, 3, 4, and 7 days after inoculation (dai) were mounted directly onto aluminum stubs without fixation. They were examined using the Hitachi S-4300 SE/N scanning electron microscope (Hitachi High Technology America, Pleasanton, CA) at 10 to 20 kV in the variable pressure mode. Digital images were acquired using the image processing system in the instrument. The number of germ tubes was counted from 83 germinated conidia. Means and standard errors of hyphal length between initial and secondary appressoria and between lateral appressoria were calculated using 44 and 16 digital images, respectively.

Light microscope examination. Leaf segments (0.5 by 0.5 cm) were cut from inoculated leaf disks and fixed in 50% FAA solution (5 ml of 37% formaldehyde, 5 ml of glacial acetic acid, 50 ml of 95% ethyl alcohol, and 40 ml of water) 2 weeks after inoculation. After a minimum of 24 h of fixation period, leaf samples were dehydrated using a graded series of isopropyl alcohol for 30 min and then were infiltrated with paraffin (Paraplast Plus Tissue embedding medium; Oxford Labware, St Louis) in a 60ºC oven overnight. Samples were embedded in paraffin and 12-µm sections were cut using a rotary microtome (Reichert-Jung 820-II HistoCut; Nussloch, West Germany). Sections were mounted on slides and the paraffin removed using Microclear (Micron Environmental Industries, Fairfax, VA). The sections then were rehydrated in a graded ethanol series and stained with 0.6% Coomassie brilliant blue R-250. Samples were examined under a light microscope. Time course of spore germination and appressorium formation on glass slides was recorded under a light microscope at 1, 2, and 12 hai and 2, 3, and 7 dai, respectively. Images of light microscope observation were acquired using a Sony digital still camera MVC-CD500 (Sony Electronics Inc., Park Ridge, NJ). A conidium was defined as having germinated if the conidium formed a germ tube with an initial appressorium or the germ tube was at least as long as the width of the conidium.

RESULTS

Germination of conidia. Conidia began germinating within 2 hai on both leaf disks and glass slides (Table 1; Fig. 1A–D). One to four initial germ tubes grew from the two poles of a conidium (Fig. 1C and D); one or two germ tubes per conidium were common (94%) and three to four germ tubes were less frequent (6%).

Formation of initial and secondary appressoria. Initial appressoria were differentiated from one or two of the germ tubes on a conidium (Fig. 2A). However, the formation of the initial appressoria was

delayed on glass slides compared with leaf disks (Table 1). Initial appressoria began to form within 3 hai on leaf surfaces (Fig. 2A), whereas initial appressoria formed at 48 hai on slides (Fig. 2D). Initial germ tubes were longer on glass slides than on leaf disks (Fig. 2A–D). Hyphae grew from only one of the germ tubes with initial appressoria on a germinated conidium on both glass slides and leaf disks (Fig. 2C–F). Development of hyphae and formation of secondary appressoria were observed on leaf disks 12 and 24 hai, respectively (Fig. 2C and E). On glass slides, however, growth of hyphae from initial appressoria and formation of secondary appressoria were observed 3 dai (Fig. 2F). The initial and secondary appressoria developed singly or in pairs opposite one another, and appeared lobed (Fig. 2A, C–F).

Growth of branched hyphae and sporulation. Branched hyphae did not grow from germinated conidia even 7 dai on glass slides (Table 1) and, in most instances, it appeared that hyphal growth had stopped and hyphae had collapsed. On leaf disks, branched hyphae and lateral appressoria were observed 2 dai (Fig. 3A). Branched hyphae grew from the hyphae between initial and secondary appressoria and directly from secondary appressoria. Hyphae radiated from only one of the germ tubes on a germinated conidium (Fig. 3B–D). The shape and size of lateral appressoria on branching hyphae were similar to those on germ tubes. However, the length of hyphae between the lateral appressoria (71.54 ± 6.63 µm; mean ± standard error [SE], n = 16) was longer than that between the initial and secondary appressoria (35.11 ± 1.02 µm; mean ± SE, n = 44) (Fig. 3A–D). At 7 dai, conidia developed on short conidiophores displaying a twisted basal cell (Fig. 3D and E). Light microscope examination of infected leaf sections revealed that globose haustoria in host epidermal cells were connected by haustorial necks to appressoria on the surface of the host epidermal cells (Fig. 3F).

DISCUSSION

Conidia of *E. pulchra* germinated and formed initial and secondary appressoria on both leaf disks and glass slides. These results indicate that conidia of *E. pulchra* have the potential for germination, appressorium formation, and limited hyphal growth without establishing a parasitic relationship with host cells. However, for-

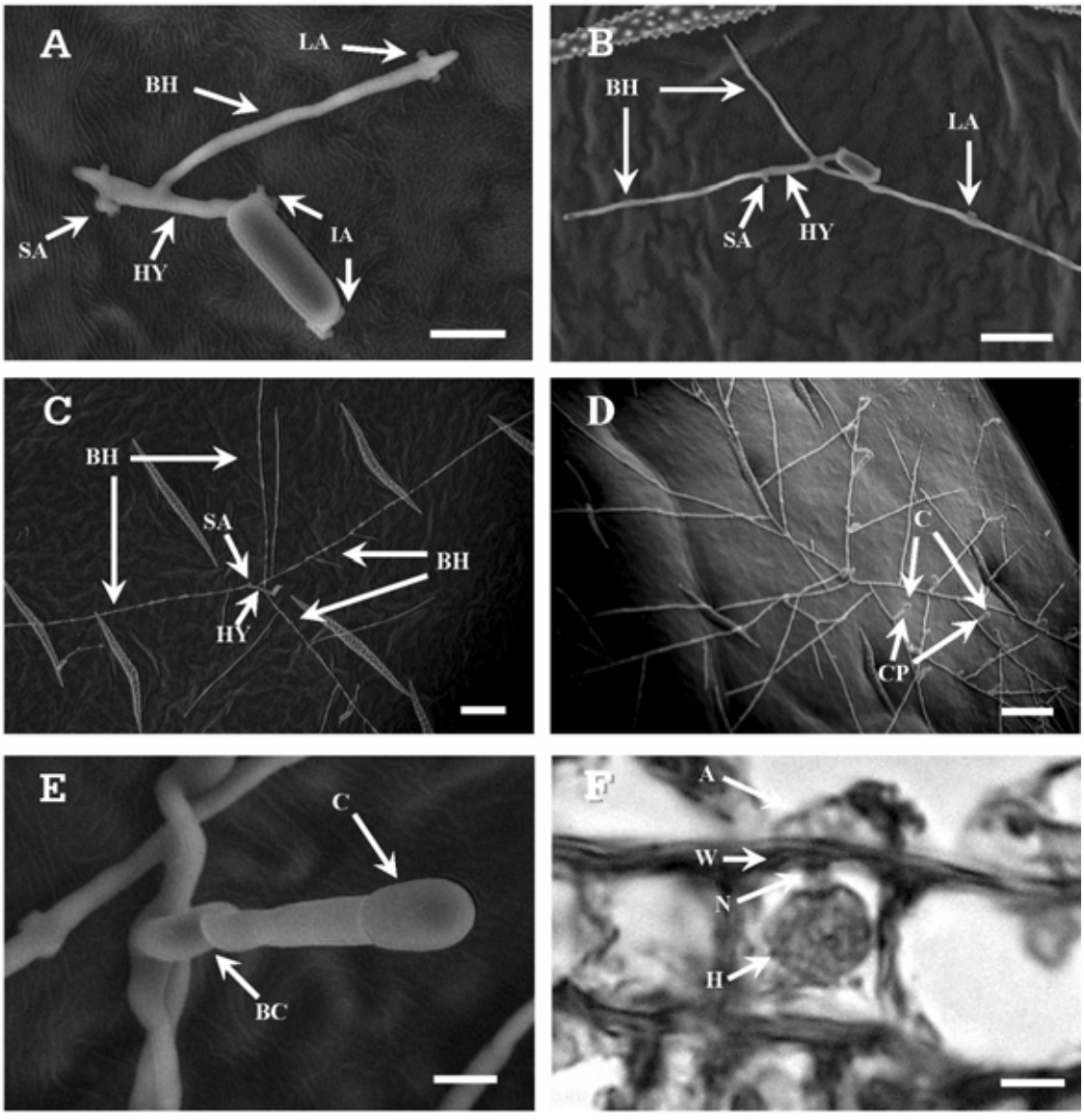

Fig. 3. A–E, Scanning electron micrographs of branching hypha growth on leaf surface and **F,** light micrographs of a haustorium of *Erysiphe pulchra* in a leaf cell. **A,** Lateral appressorium (LA) and branched hypha (BH) emerging from hypha (HY) between the initial appressorium (IA) and secondary appressorium (SA) 2 days after inoculation (dai). Bar = 20 µm. **B,** BH from HY and from secondary appressorium 3 dai. Bar = 100 µm. **C,** Growth of BH from HY and secondary appressorium 4 dai. Bar = 200 µm. **D,** Conidia (C) formation on conidiophores (CPs) in a colony 7 dai. Bar = 200 µm. **E,** Close up of conidium and CP with arched basal cell (BC). Bar = 10 µm. **F,** Globose haustorium (H) in a host epidermal cell and the haustorial neck (N) connecting haustorium (H) body and appressorium (A) on the surface of epidermal cell wall (W). Bar = 5 µm.

mation of initial and secondary appressoria was delayed and branched hyphae failed to form on glass slides. Earlier formation of initial and secondary appressoria on leaf disks than on glass slides is most likely because the topography of the leaf surface structure stimulated the formation of appressoria. In barley (*Hordeum vulgare* L.), the physical structure of a wax layer on the leaf surface could stimulate the formation of normal and mature appressoria of *B. graminis* (5).

Resources are conserved when germ tube growth is limited before formation of appressoria (7). In *E. pulchra*, ontological events support this hypothesis. The shorter hyphae between the initial and secondary appressoria than that between lateral appressoria indicate that energy is limited when the conidium is the source of nutrition. For barley, the elongated germ tube (from which elongating hyphae formed) of *B. graminis* was considered evidence that the fungus had established a functional nutritional relationship with the host (5). Similarly, Ficke et al. (6) reported that formation of secondary hyphae was evidence of successful establishment of the *Uncinula necator* (Schwein.) Burrill on grape (*Vitis vinifera* L. and *V. labruscana* L.). Branched hyphae formation from germinated conidia on leaf disks can be used as a sign of successful establishment of the host–parasite relationship and as a characteristic of host–pathogen interaction in the early stage of the infection process of *E. pulchra* on dogwood.

Haustoria are thought to be the conduit for transmitting nutrients from the host to the pathogen and considered as the interface establishing the host–parasite relationship (8). Two types of haustoria have been described for powdery mildews (9). Both types have an ellipsoidal central body; however, unlike the straight finger-like branches considered to be the unique feature of *Blumeria* spp., other powdery mildews extend convolutedly over the surface of the body (2,3). In the present study, haustoria of *E. pulchra* were globose compact structures with rough surface that could be due to coiled branches on the haustorium body.

Time required for germination of conidia in this study was similar to that reported for other powdery mildew fungi (4,11,13,14). However, formation of appressoria and development of hyphae differed from other powdery mildew pathogens. For *B. graminis* on barley, the initial germ tubes did not form appressoria, but the secondary germ tubes formed hook-shaped appressorial lobes and hyphae developed from appressoria (1,8,11). In contrast, formation of primary appressoria on the initial germ tubes and development of hyphae from both initial and secondary germ tubes were reported for *E. pisi* (13,14), *S. fuliginea* (12), and *O. lycopersicum* (10). In the present study, for *E. pulchra*, initial appressoria formed from one or two germ tubes and branched hyphae grew from only one of the germ tubes with initial and secondary appressoria. In comparison with some species of *Erysiphe*, formation of primary germ tubes was considered a unique and characteristic feature of *B. graminis* development (11). Therefore, it seems most likely that patterns of infection structure formation and branching hyphal growth are distinguishable in different pathosystems.

In the present study, we described in detail the development time course for *E. pulchra* on susceptible dogwood leaf disks and on glass slides. Further studies on the penetration and haustorium formation of the pathogen and defense response to *E. pulchra* are needed using dogwood cultivars with different levels of resistance.

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