

Infection Process of *Sphaerotheca fuliginea* on Host Cucumber and Nonhost Wheat Leaves

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Abstract

The infection process of *Sphaerotheca fuliginea* into epidermal cells of host cucumber and nonhost wheat were studied histochemically. The percentages of germinated conidia had no significant difference on cucumber and wheat leaves at the pre-penetration stage. When *S. fuliginea* overcame the constitutive barriers of wheat leaf, papillae and hypersensitive reaction were induced to prevent the further development of *S. fuliginea*. Less than 1% haustoria could form in wheat at the post-penetration stage. However, in cucumber to *S. fuliginea* interaction, plentiful secondary hyphae and haustoria formed in cucumber leaves.

Introduction

Cucumber powdery mildew (*Sphaerotheca fuliginea*) is a severe greenhouse disease spreading worldwide and results in significant yield losses (7). *S. fuliginea*, absorbing nutrition from its host, is an obligate parasite, and its host range is very narrow. Why not colonize nonhost plants? What are their resistance mechanisms?

In recent years, much progress has been made to elucidate the mechanisms of nonhost disease resistance by utilizing biochemical, pharmacological and microscopical methods as well as functional genomic technologies (2, 9). The resistance responses of nonhost include cytoplasmic aggregation, papilla formation, accumulation of reactive oxygen species (ROS), defense-related autofluorescent materials and organelles present at around fungal penetration sites (4, 8).

Compared with other pathogens, powdery mildew fungi possess particular advantages for the study of their infection process on host and nonhost plants. Being external plant surfaces parasites, their conidium germination, appressorium and haustorium formation, in addition, hyphal growth and certain defense reactions, can be easily observed under a light microscope. The aim of this study is to obtain the preliminary cognition of

infection process of *S. fuliginea* on host cucumber and nonhost wheat.

Materials and Methods

The cucumber (*Cucumis sativus* L.) 'Changchun Thorn' and wheat (*Triticum aestivum* L.) 'Shuiyuan 11', were grown from seed in organic soil in a growth chamber under fluorescent lights (60 mmol/m²/s photon flux density), 60-70% relative humidity (RH) and 12 h photoperiod at 25°C for cucumber and 12 h photoperiod at 20°C for wheat. Third leaves of one-month-old seedlings of cucumber and primary leaves of seven-day-old seedlings of wheat were used for inoculation.

Cucumber powdery mildew fungus was maintained on the susceptible cucumber cv. 'Changchun Thorn'. For inoculation, freshly collected conidia were applied with a fine paintbrush to the upper surface of the cucumber and wheat leaves at a density of 20 conidia mm⁻², and inoculated leaves were incubated in a growth chamber at 20° ± 1°C, 100% RH in the dark for 12 h followed by incubation at 70-80% RH and a photoperiod of 12 h : 12 h (L:D) (60 mmol/m²/s photon flux density).

The inoculated leaves were sampled at the indicated times and decolorized in boiling 95% ethanol. Then the tissues were clarified in saturated chloral hydrate overnight. Hypersensitive cell death was detected using the trypan blue staining method (3). At the indicated times, the inoculated leaf segments were boiled in a mixture of phenol, lactic acid, glycerol and distilled water containing 1 mg/mL trypan blue (1:1:1:1) for 3 min. Then the leaf segments were clarified overnight in saturated chloral hydrate before being stored in 50% glycerol.

For microscopical observation, the leaf tissues were rinsed in distilled water for 5 min before they were stained with an aqueous solution of 0.05% coomassie brilliant blue (Bio Basic Inc., Shanghai, China) for 5 min (3). Germination was defined when the length of germ tube was over half of maximum diameter of conidium. The treated leaf pieces were mounted on glass slides in 50% glycerol before

observation using an Olympus CX21FS1 light microscope and a Nikon 50i microscope equipped with a differential interference contrast (Japan).

Results

On cucumber leaves, the conidia of *S. fuliginea* germinated and formed 2-4 germ tubes, and the majority of conidia formed three or four germ tubes (Fig. 1A). At 8 h after inoculation (hai), most of conidia had germinated on cucumber epidermal cells, and the appressoria began to develop at the first germ tube tip. At the middle of appressoria formed primary haustoria, then primary haustoria could absorb nutrition from cucumber and develop other germ tubes, followed by the formation of elongated secondary hyphae and secondary haustoria. With the development of hyphae, clusters of conidia were detected under a light microscope and colonies were visible on the surface of cucumber leaves with naked eyes. On cucumber leaves, only a few of abnormal appressoria could be detected at 48 hai.

On wheat leaves, the conidia of *S. fuliginea* began to germinate at 10 hai, the germination of conidia on wheat was later than on cucumber, but the germination rates on wheat and cucumber leaves had no significant difference from 36 hai (data not shown). Compared with on host cucumber, the germinated conidia formed one to two germ tubes on nonhost wheat leaves, and most of them had only one germ tube (Fig. 1B). At 18 hai, the majority of germ tubes had formed matured appressoria. Some appressoria were two lobed or slender irregular appressoria. Thickened cell wall, papillae and hypersensitive cell death were observed at penetration sites of *S. fuliginea* (Fig. 1B, D). Germinated conidia rarely formed primary haustoria (<1%), and the haustoria were shriveled and elongated abnormally. No hyphae and secondary haustoria were observed on wheat leaves.

Discussion

The infection process of *S. fuliginea* on host cucumber and nonhost wheat was studied in this paper. The appressoria of conidia formed at similar rates on cucumber and wheat leaves, indicating that no resistance was expressed during the pre-penetration stage of *S. fuliginea*. The constitutive barriers, including wax layers, cell walls and antimicrobial secondary metabolites are the first line of plants against the pathogens (1, 5, 6, 10). Appressoria developed penetration pegs, from which secreted one or more sets of enzymes to degrade the cell walls, and tried to penetrate into cucumber and wheat cells. When *S. fuliginea* overcame constitutive barriers, it would face the intracellular defence response. Recent studies have emphasized the

importance of host receptors for pathogen-associated molecular patterns (PAMPs) in host and nonhost resistance (12). Compare with nonhost and resistant plants, PAMP-induced defense in susceptible plants is deficient in preventing infection of pathogens (11). Therefore, the nonhost resistance exhibited by wheat towards *S. fuliginea*, such as cytoplasmic aggregation, papilla formation and hypersensitive cell death et al., resulted in cessation of *S. fuliginea* growth. Further study is required to elucidate the mechanisms in expression of nonhost resistance at molecular level.

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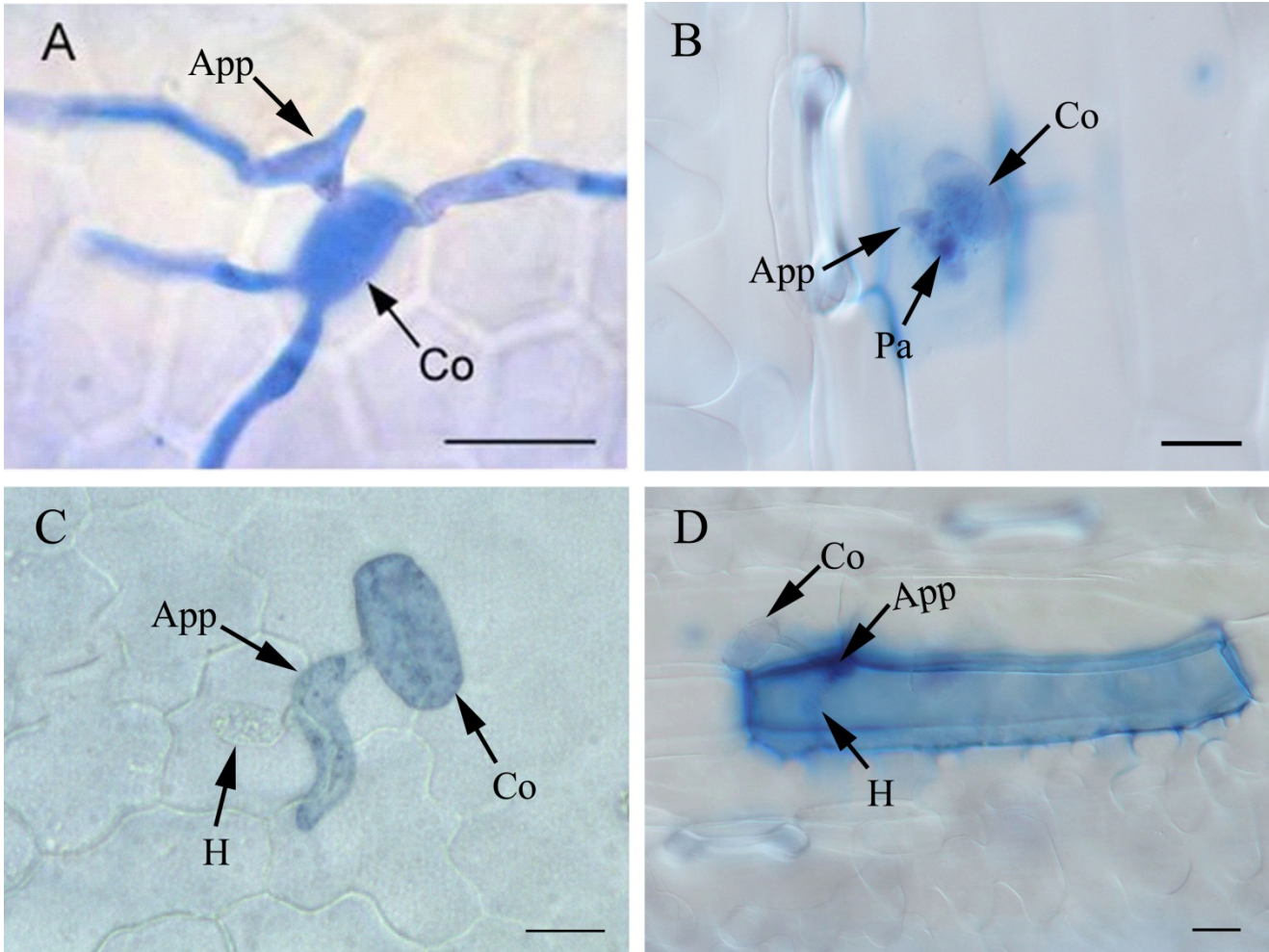


Fig. 1. Light micrographs showing of penetration by *S. fuliginea* in host cucumber (A, C) and nonhost wheat (B, D). Germinated conidium is evident by coomassie brilliant blue staining. (A) Conidium germinates with four germ tubes on cucumber leaf, 48 hai. (B) Conidium develops an appressorium on wheat, and defence reactions are shown by wheat against *S. fuliginea* with papilla formation and trypan blue staining in anticlinal cell walls, 18 hai. (C) At the middle of appressorium forms a plump rounded haustorium, 18 hai. (D) Shriveled and elongated haustorium forms in wheat epidermal cell with hypersensitive cell death, 48 hai. App, appressorium; Co, conidium; H, haustorium; Pa, papilla. Bars=20µm.