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The Pathogenicity of *Sclerotium rolfsii* on *Cyperus difformis* and its Potential Host Specificity among the Genus *Cyperus*

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Abstract

Sclerotium rolfsii Sacc. infects more than 500 species of monocotyledonous and dicotyledonous plants except Cyperaceae family. The pathogenicity of a *S. rolfsii* isolate was evaluated by seven *Cyperus* species in order to explicate host specificity to Cyperaceae family. The results showed that only C. dofformis L. was infected with typical water-soaked lesions of the basal stem, which progressed to rotting, wilting, blighting, and eventually death. The performance of hyphae on the surface of *Cyperus* plants was compared and found that only stomata of *C. difformis* were adhered by hyphae of *S. rolfsii*. The infection process of *S. rolfsii* on leaf sheath of stem base in *C. difformis* showed that dense mycelial networks and ramifying hyphae were usually formed on the inoculated tissues, then growing hyphal tips were observed to spread wavelike on the stem surface, reaching the stomata between the leaf veins accurately and directly enter the host through stomata. Differences of the main micro-morphology characters of leaf sheath abaxial epidermis among the seven species were compared. The stomata of *C. difformis* were always presented between the leaf veins (3 or 4 rows of cells from the leaf veins), while the stomata of tolerant *Cyperus* species were close to the leaf veins. Underneath the stromata of *C. difformis* were air chambers, however vascular bundles were always present underneath the stomata of the tolerant *Cyperus*. Our study indicates that different anatomical structures in genus *Cyperus* may be associated with resistance to *S. rolfsii* infection.

Keywords: *Sclerotium rolfsii* isolate SC64; *Cyperus difformis*; Stromata; Transverse section; Host specificity; Infection process

Introduction

Sclerotium rolfsii is a versatile soil borne pathogen commonly occurs in the tropics, subtropics, and other warm regions, especially at high humidity and warm temperatures. It may cause a variety of diseases, for example, damping off of seedlings, collar or stem rot, foot rot, crown rot, *Sclerotium* wilt and blight [1]. Previous studies have reported that *S. roflsii* infects more than 500 species of monocotyledonous and dicotylednous plants, especially severe on vegetables, flowers, legumes, cereals, forage plants and weeds [2,3]. The signs and symptoms of Sclerotinia minor were observed on yellow nutsedge (*Cyperus esculentus L.*) in Bertie County [4], but infection of *S. rolfsii* in *Cyperus* spp. has not been reported yet.

The histopathology of infection by *Sclerotium* spp. has been studied in considerable detail. Previous histopathological studies have reported that *S. rolfsii* penetrates host tissue by formation of appressoria [5,6], followed by apparent tissue necrosis in advance of the mycelium [6]. Phytotoxins such as oxalic acid and cell wall degrading enzymes play a key role in the infection of a host [1,7] and a multi-enzyme system for the degradation of different polysaccharides was discovered in the host tissue [8]. Although "hyphal aggregates" have been reported to form during infection by *S. rolfsii*, the role of these aggregates in pathogenesis has not been determined, and tissue death in advance of mycelial growth has also not been conclusively demonstrated.

S. rolfsii isolate SC64, a fungus indigenous in Jiangsu province, was isolated from an alien invasive weed *Solidago canadensis L.* (Canadian goldenrod, *Asteraceae*) [9]. The fungus caused basal stem rot lesions on *S. canadensis* and was found capable of controlling some dicotyledon weeds and *Cyperus difformis*, which was unrecognized as the host before, in a host range test and field trials [10]. Understanding the infection differences between *C. difformis* and other species of the *Cyperus* family to *S. rolfsii* isolate SC64 is imperative to estimating the host range of this isolate as a biocontrol agent and enriching the infection mechanism of *S. rolfsii*.

Therefore, the objectives of this research were to 1) determine the host specificity of *S. rolfsii* isolate SC64 among 7 species in genus *Cyperus* 2) study in detail the performance of *S. rolfsii* isolate SC64 on *C. difformis* stem surface by using light and scanning electron microscopy and 3) compare the transverse section of *C. difformis* with six other *Cyperus* species by using paraffin section observation and try to elucidate the selective mechanism of *S. rolfsii* among *Cyperus* spp. based on anatomical structure differences.

Materials and Methods

Pathogen and host plant: Isolate SC64 of *S. rolfsii* from S. canadensis in Nanjing city, Jiangsu province of China, was used throughout this study. For inoculum preparation, the fungus was grown in Petri dishes on potato dextrose agar (PDA) at 28°C in the dark for 2-3 days.

Greenhouse-grown host plants included in this study were *C. difformis* L.; *C. rotundus* L.; *C. iria Linn.*; C. *glomeratus L.*; C. *amuricus Maxim.* and C. *cuspidatus* H.B.K. Plants were transplanted from wild field (near a lake in Xiamafang Park, Nanjing city) into plastic pots (14 cm in diameter) and grown in the greenhouse under natural photoperiod and temperature conditions.

Host specificity tests: A starter culture of isolate SC64 was produced by placing five agar plugs (5 mm diameter), cutting from the actively growing margin of the PDA culture, into 100 ml (250 ml flask) potato dextrose broth (PDB, potato extract, 20 g D-glucose and water to

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make 1 L, pH 5.0). The starter culture was cultured (28°C) in an orbital shaking incubator (110 rpm) for 7d. and aseptically blended. The starter culture was then used to inoculate plants growing in pots. Each pot contained three plants and each species was replicated four times. The experiments were performed twice. Each plant was inoculated with 0.5 ml of starter culture to the basal stem by 1 ml pipettor. After inoculation, plants were moved to greenhouse with natural light and temperature of 25~35°C. The soil was maintained wet throughout the experiment by adding water to saucer under the pots. Control plants were inoculated with autoclaved water only. Plants were monitored every day for two weeks to detect the characteristic symptoms of basal stem water soaked lesions and wilt caused by *S. rolfsii*. Monitoring was conducted to record the presence or absence of these symptoms.

A dose response test of *C. difformis* to *S. rolfsii* SC64 was also carried out. Seedlings with 3.5~5.5 leaf stages were inoculated with fresh fungus-infested cotton seed hulls at 60~120 g·m^{-2}. Plant mortality and fresh weight reduction were evaluated 14 days after inoculation. Surviving plants were excised at soil surface level, weighed and the percentage of biomass reduction was determined as compared to the control plants. The experiment included four replications for each treatment and was repeated once. The control treatment was treated with autoclaved cotton seed hulls.

Inoculation of stems of *Cyperus*: Mature *Cyperus* plants stem were excised at the soil surface level, rinsed in tap water and blotted dry. Leaves were peeled off carefully from the stems and placed on moistened filter paper in Petri dishes (9 cm in diameter). Tissues were inoculated with agar disks (5 mm diameter) cut from the advancing margin of 3-day old cultures of the pathogen grown on PDA. Dishes were sealed in polyethylene bags and incubated at 25~28°C with a 12-hr photoperiod under cool-white fluorescent lights.

Light microscopy: At approximately 4-hr time intervals from 12 to 32 hr after inoculation, pieces of infected tissues from appropriate regions were fixed for 24 hr in FAA (70% ethanol：38% formaldehyde：glacial acetic acid, 90:5:5, v/v/v). The growth of *S. rolfsii* SC64 was estimated by lactophenol aniline blue staining as describe previously (Govrin and Levine, 2000). The other part of uninoculated stem of the 7 *Cyperus* species was fixed in FAA for observing the surface morphological characteristics and transverse sections with light microscopy. The transverse sections of stems were made through usual paraffin method in thickness of 8 µm, and stained with safranine and fast green. The pictures were taken using the image analysis software Motic Images Plus version 2.0.

Scanning electron microscopy: Leaf and stem Samples from C. dofformis were taken 8, 12, 16, 20 and 24 hr after inoculation (hai). Samples were first fixed with 4% (v/v) glutaraldehyde in 50 mM phosphate buffer (pH 6.8) for 8~10 hr at 4°C, then rinsed with the same buffer for 3 hr. After dehydration in a graded acetone series, the samples were critical-point dried, mounted on stubs, sputter coated with gold-palladium, and viewed using a HITACHI S-3000 scanning electron microscope operating at 15 kV.

Results

Host specificity and pathogenicity to *Cyperus difformis*: Two days after inoculation of *S. rolfsii*, characteristic lesions were observed on the basal stems *C. difformis*. Leaves collapsed 2~5 days after inoculation, and then 2~4 cm basal stem rot lesions appeared and the whole plant began to wilt and die. Meanwhile white sclerotia appeared around the basal stem and soil surface and quickly turned brown in 1~2 days (Figure 1). Infested tissues and mature scleotia were collected for re-isolation of the fungus. Microscopic examination and culture of isolate from *C. difformis* confirmed that it had been infected by *S. rolfsii* SC64. None of other plants inoculated with *S. rolfsii* showed any signs of pathogenicity and therefore are considered to be immune to this pathogen (Table 1). Estimated parameters of fitted logistic equation in dose response test showed that LD50/90 were 79.8/122.7 g·m^{-2} and 64.4/113.4 g·m^{-2} for plant mortality or fresh weight reduction of *C. difformis* when treated with *S. rolfsii* SC64-infested cotton hulls (Table 2).

Performance of *S. rolfsii* SC64 hyphae on *Cyperus* stems by Light microscopy: In order to further understand how the *S. rolfsii* isolate

Figure 1: Symptoms of stem rot on *Cyperus difformis* L. caused by *Sclerotium rolfsii* isolate SC64. A, Healthy plants; B, Typical symptoms on stems and near the soil line after 2 dai; C, Typical symptoms of leaves wilt after 2 dai; D, plant mortality after 10 dai.

Cyperus Species	Rating for *S. rolfsii* SC64
C. amuricus Maxim.	–
C. cuspidatus H.B.K.	–
C. rotundus L.	–
C. glomeratus L.	–
C. iria L.	–
C. compressus L.	–
C. difformis L.	+

Note: "+" means shows infection.

Table 1: Plants included in host-specificity testing of *S. rolfsii* isolate SC64.

Efficacy (%)[a]	Dosage (g·m^{-2})					Regression equation	LD_{50}/g·m^{-2}	LD_{90}/g·m^{-2}
	0	60	80	100	120			
PM	0e	33.3d	51.7c	66.7b	87.7a	y=44761.1-44761.1/(1+(x/11540.47)^1.37) (R^2=0.9958)	79.8	122.7
FW	0e	42.4d	71.3c	85.2b	91.6a	y=97.10-97.1/(1+(x/63.56)^4.39) (R^2=0.9994)	64.4	113.4

[a] MR: Mortality Rate (%), FW: Fresh Weight Reduction (%). Means within the same row followed by different letters are significantly different at P<0.05 level according to Duncan's multiple-range test.

Table 2: Effect of fungus-infested cotton hulls of *S. rolfsii* SC64 application dosage on plant mortality and fresh weight reduction of *C. difformis*.

SC64 may differentially infect its hosts, in this work we demonstrated the performance of hyphae on the hosts stem surface. Temporal analysis of the fungal structures upon infection of *Cyperus difformis* with the *S. rolfsii* isolate SC64 via aniline blue staining indicated that, at 12 hr post inoculation, hyphae of *S. rolfsii* SC64 ramified over the surface of all inoculated tissue (Figure 2a). Hyphae frequently ramified towards the stomata (Figure 2b-2d), where more intense staining was found. Ramified hyphae or adhering upon stomata were not observed on the non-susceptible *Cyperus* species (Figure 2e and 2f).

The infection process on *C. difformis* stem surface by *S. rolfsii* SC64: The scanning electron microscopy (SEM) observations showed that the running hyphae grew from the inoculum disks over the stem surface and formed a dense hyphal network, especially between the leaf veins (Figure 3a) within 12 hai. The host surface was covered by the ramifying hyphae, which were relatively smaller (Figure 3b). Growing hyphal tips on the root surface were also observed to spread wavelike on the stem surface, reaching the stomata between the leaf veins accurately (Figure 3c and 3d). Slime material (mucilage) covering hyphae, hyphal tips and extending between the hyphae, was also deposited on the plant surface. Small changes in cuticle integrity were observed (Figure3f) and the infection hyphae entered host tissue through the open stomatal (Figure 3e). Appressorium structure was also observed on the stomata. Water soaked lesions were visible in the leaf and stem parts which underneath the inoculum disks and the host tissue turned soft at this developmental stage. Hyphae also penetrated the host surface directly through cracks. The host surface was depressed and penetrating hyphae grew into the host tissue (Figure 4g and 4h).

The comparison of the main micro-morphology characters of basal leaf abaxial epidermis among 7 species of *Cyperus*: The basal stem leaf abaxial epidermis structures of 7 species of the genus *Cyperus* were studied under light microscope. The results (Table 3) showed that the genus *Cyperus* was highly consistent in the micro-morphology

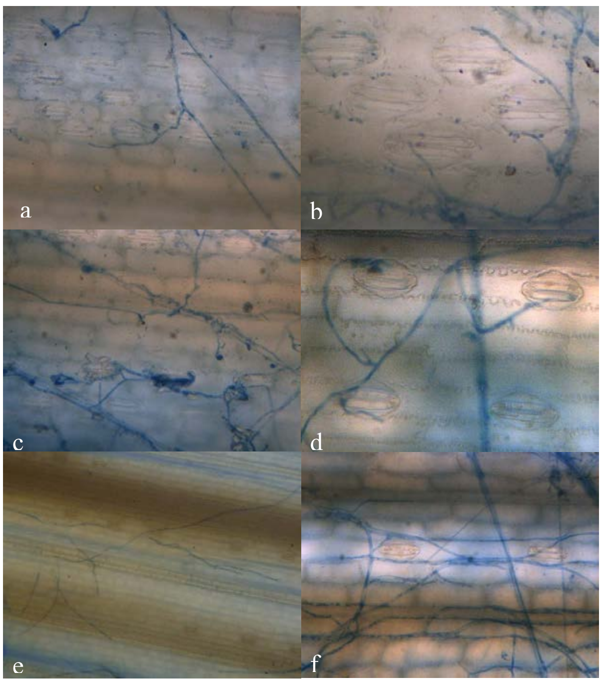

Figure 2: The performance of *Sclerotium rolfsii* isolate SC64 on surface of *Cyperus difformis* (a~d, 40×) and non-susceptible *Cyperus* species. (e *C. rotundus* L., 20×; f *C. iria* L.,40×).

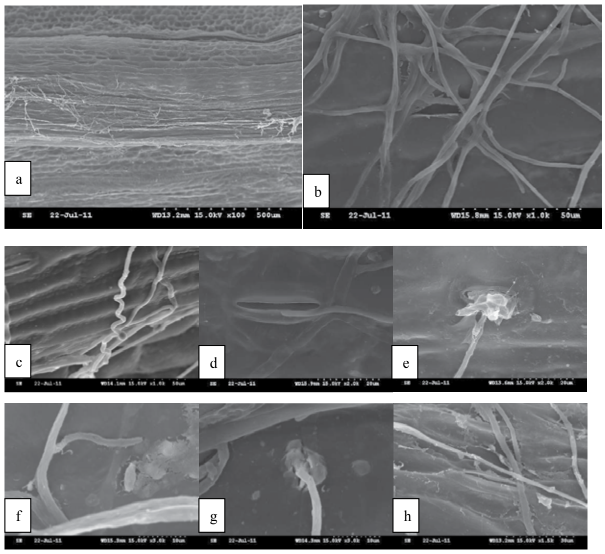

Figure 3: Development of *Sclerotium rolfsii* isolate SC64 on stem of *Cyperus difformis* observed with scanning electron microscopy (a) Hyphae of *S. rolfsii* SC64 on the leaf surface which formed a dense hyphal network, 100×; (b) Branching hyphae of *S. rolfsii* SC64, 1000×; (c, d, e) Wavelike hyphae spreading on the leaf surface, secreting mucilage and entered into the host tissues through the stromata, 1000×; 2000 × and 2000×, respectively; (f, g, h) Mucilage covering hyphal tips and ramifying hyphea entered into the host tissues through crevasses on the stem surface, 3000×; 3000 × and 1500×, respectively.

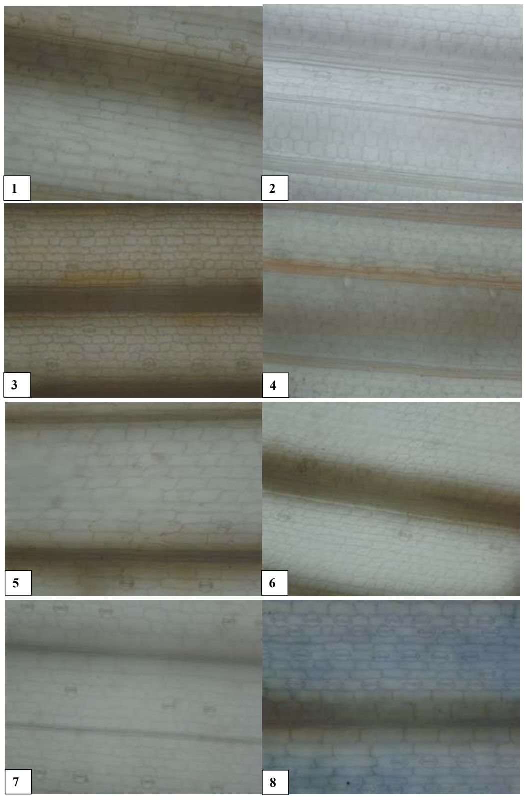

Figure 4: Microphotographs of basal stem leaf abaxial epidermis under L M (20×, dark lines in the pictures were leaf veins). 1. *C. amuricus* Maxim.; 2. *C. compressus* L.; 3. *C. cuspidatus* H.B.K.; 4. *C. glomeratus* L.; 5. *C. iria* L.; 6. *C. rotundus* L.; 7. *C. difformis* L.; 8. *C. difformis* L. (upper leaves).

Species	Shape of long-cells between veins	Short cells	Place that stomata present	Number of stomata under 20× microscopic ocular	Length/width of stomata)	Shape of subsidiary cells	Place that papilleas present	Shape of guard cells
C. amuricus Maxim.	Long-tubular, simuous	Absent	Close to the leaf veins (2 or 3 rows of cells from the leaf veins)	3	1.27	Dome-shaped	Over the veins	Both sides not obviously thicker
C. cuspidatus H.B.K.	Long-tubular, simuous	Absent	Close to the leaf veins (2 or 3 rows of cells from the leaf veins)	7	1.06	Dome-shaped	Over the veins	Both sides obviously thicker
C. rotundus L.	Long-tubular, simuous	Absent	Close to the leaf veins (2 or 3 rows of cells from the leaf veins)	4	1.33	Dome-shaped	Over the veins	Both sides obviously thicker
C. glomeratus L.	Short-tubular to long-tubular, simuous	Absent	Close to the leaf veins (2 rows of cells from the leaf veins)	5	1.56	Dome-shaped	Over the veins	Both sides not obviously thicker
C. iria L.	Short-tubular to long-tubular, simuous	Absent	Close to the leaf veins (2 or 3 rows of cells from the leaf veins)	2	1.06	Dome-shaped	Over the veins	Both sides not obviously thicker
C. compressus L.	Short-tubular, rarely sub-terragonal, deeply simuous	Absent	Close to the leaf veins (2 rows of cells from the leaf veins)	5	1.66	Dome-shaped to triangular	Over the veins	Both sides obviously thicker
C. difformis L.	Short-tubular, rarely sub-terragonal, deeply simuous	Absent	Between the leaf veins (3 or 4 rows of cells from the leaf veins)	7	0.99	Dome-shaped to triangular	Over the veins	Both sides obviously thicker

Table 3: The comparison of the main micro-morphology characters of leaf abaxial epidermis of 7 species of *Cyperus*.

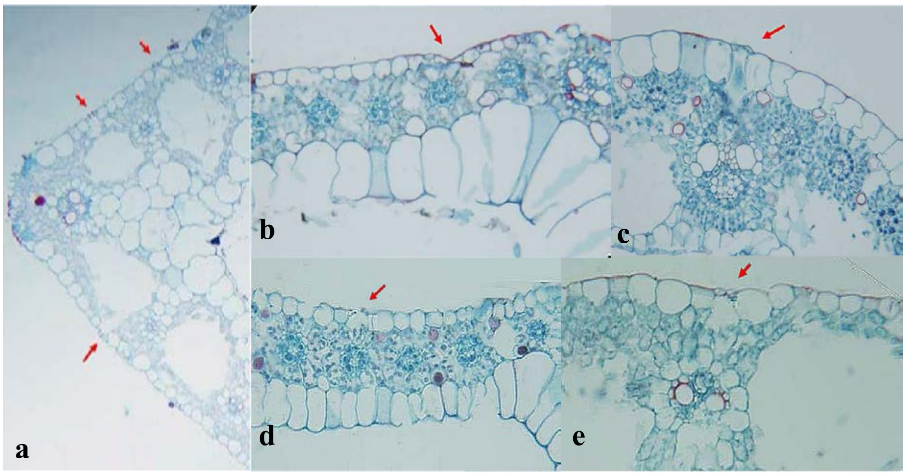

Figure 5: The transverse section of some *Cyperus* species (arrow shows the place of stomata, 40×) (a *C. difformis* L.; b *C. cuspidatus* H.B.K.; c *C. iria* L.; d *C. glomeratus* L.; e *C. amuricus* Maxim.).

characters, e.g. the shape of long-cells long-tubular or short-tubular, rarely sub-tetragonal, the margin of cell walls sinuous or deeply sinuous; short cells absent; stomatal subsidiary cells triangular, dome-shaped to triangular, tall dome-shaped and dome-shaped; papillaes present over the veins (Figure 4). However, the presence, shape, and distribution of stromata in the epidermal surface were different from other six *Cyperus* species. The stomata of *C. difformis* were always present between the leaf veins (3 or 4 rows of cells from the leaf veins). The density of stromata was relatively higher and the length/width of stomata was relatively lower than other six *Cyperus* species. We propose that the micro-morphological characteristics of stromata may play an important role in the fungal infectivity.

The comparison of transverse section of *Cyperus* species: The differences of stromata characteristics between *C. difformis* and the non-susceptible *Cyperus* species were visually apparent in the examination of basal leaf cross-sections (Figure 5). Vascular bundle was always present underneath the leaf veins. The stromata of the non-susceptible *Cyperus* species were always close to the leaf veins, so under the stromata were mesophyll cell, bundle sheath cell and vascular bundle. However, the case was different in *C. difformis*, where the stromata presented in the middle of vascular bundles and below the stromata was air space. From this point of view, the structure of *C. difformis* was more beneficial for fungus infection.

Discussion

Sclerotium rolfsii is a polyphagous pathogen in the world and new record of host species are reported continually, which includes some monocotyledonous plants e.g. *Poa annua* L. [11], garlic (*Allium sativum*) [12]; Asiatic dayflower (*Commelina communis L.*) [13]. Our experiment on the stem rot of *C. difformis* caused by *S. rolfsii* contributes one more new host specie for this fungus. Further studies on the fungus have shown that it is an effective agent of biological control of the weed in dry direct-seeded rice [10].

In the host specificity test of our study the primary nutrient sources of *S. rolfsii* was mycelium grown from PDB medium. Hyphae growing from the PDB suspension extended and ramified on the stem surfaces. However, there is substantial hyphae growth and extension difference under the light microscopy. Only the atomata of *C. difformis* were adhered by ramified hyphae of *S. rolfsii*. The pathogen directly penetrates the host surface via stomata. Therefore, stomata play an important role for *S. rolfsii* infection of *C. difformis*. However, stomata-penetrating pathogens need appropriate cues to locate stomata pores [14]. The development of wavelike hyphae may be assumed to increase the adhesion of the pathogen to the host surface in order to effectively reach the stomata. Leaf veins located close to stomata of the resistant *Cyperus* species might a natural fence, making a false angle for the eruptive hyphae.

Infection cushions are produced by many plant pathogenic fungi (e.g. *Sclerotinia sclerotiorum*; *Rhizoctonia solani*) and these structures were reported to facilitate infection of the host [15-17]. Previous studies have reported the occurrence or presumed functions of hyphal aggregates which were formed during infection of host tissue by *S. rolfsii* [5,18]. Infection was also reported to occur from appressoria produced by germinating basidiospores of the teleomorph of *S. rolfsii* [19]. In this study, neither multicellular compound appressoria (infection cushions) nor flattened hyphae was observed on the host surface. After penetration into mesophyll via stomata, the host tissue turned yellowish-brown soft and the cuticle became disintegrated. Then hyphae entered into the host tissue through the rifts. *S. rolfsii* produce extracellular enzymes including pectin methyleasterase [7], cutinase [20], phosphatidase [21], arabanase [22], galatanase, mannanase, xylanse [23], oxalic acid and polygalacturonase [24]. It is assumed that tissue death in advance of mycelial growth during infection of *C. difformis* by *S. rolfsii*.

Plant defense against pathogen attack is complex, with many local and systemic aspects [25]. The internal anatomy and surface features of the leaves often determine plant resistance to biotrophic pathogen infection [26]. Among such characters, aspects of stromata, cuticle and trichome morphology can influence disease resistance [27]. We compared the transverse section of *C. difformis* and some other resistant *Cyperus* species and found that the variation of stomata distribution was in relation to the stem anatomy. The air chamber underneath the stomata of *C. difformis* provides the weakest mechanical obstruction to fungal penetration, while the vascular bundle underneath the stomata of resistant *Cyperus* may be a natural barrier.

In rust fungi, the emerging germ tubes adhere first to the leaf surface; subsequently, they grow and encounter stomata through directional growth [28], which in turn triggers appressorium formation [29]. Directional growth of the germ tube and formation of appressorium are controlled by the stimuli originating from the host [30]. It seems that, for the first time, an alternative 'avoidance' or pre-penetration mechanism is apparent in *Cyperus* - *S. rolfsii* interaction, which operates after the contact of parasite on the host epidermal cell [31,32]. However, we failed to observe the extension of hyphae inter- and intracellularly. More details of the infection process, the effect of mechanical obstacles of epidermis (eg. waxy deposition) and the mechanism of directional growth of hyphae all require further investigation.

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References

1. Punja ZK (1985) The biology, ecology, and control of Sclerotium rolfsii. Annual Review of Phytopathology 23: 97-127.
2. Hall R (1991) Compendium of Bean Diseases. The American Phytopathology Society, St Paul. M.N. USA, Pp 115.
3. Agrios GN (2004) Plant Pathology. (5thedn) San Diego, CA: Academic Press.
4. Hollowell JE, Shew BB (2001) Yellow Nutsedge (Cyperus esculentus L.) As a host of Sclerotinia minor. Plant Disease 85: 562.
5. Higgins BB (1927) Physiology and parasitism of Sclerotium rolfsii Sacc. Phytopathology 17: 417-448.
6. Milthorpe FL (1941) Studies on Corticium rolfsii (Sacc.) Curzi (Sclerotium rolfsii Sacc.). I. Cultural characters and perfect stage. II. Mechanism of parasitism. Proceedings of the Linnean Society of New South Wales 66: 65-75.
7. Bateman DF, Beer SV (1965) Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by Sclerotium rolfsii. Phytopathology 55: 204-211.
8. Gubitz GM, Hayn M, Sommerauer M, Steiner W (1996) Mannan degrading enzymes from Sclerotium rolfsii: Characteristics and synergism of two endo N-mannosidase. Bioresource Technology 58: 127-135.
9. Tang W, Zhu YZ, He HQ, Qiang S (2010) First report of southern blight on Canadian goldenrod (Solidago canadensis) caused by Sclerotium rolfsii in China. Plant Disease 94: 1172.
10. Tang W, Zhu YZ, He HQ, Qiang S, Auld BA (2011) Field evaluation of Sclerotium rolfsii, a biological control agent for broadleaf weeds in dry direct-seeded rice. Crop Protection 30, 1315-1320.
11. Kane KT (1992) First Report of Sclerotium rolfsii Infection of Poa annua in Illinois. Plant Disease 76: 538.
12. Kwon JH (2010) Stem Rot of Garlic (Allium sativum) Caused by Sclerotium rolfsii. Mycobiology 38: 156-158.
13. Choi O, Kwon JH, Min Y, Kim J (2011) First Report of Stem Rot on Asiatic Dayflower (Commelina communis L.) Caused by Sclerotium rolfsii in Korea. Mycobiology 39: 57-58.
14. O'Connell RJ, Panstruga R (2006) Tête à tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. New Phytol 171: 699-718.
15. Hofman TW, Jongebloed PHJ (1988) Infection process of Rhizoctonia solani on Solanum tuberosum and effects of granular nematicides. Netherlands Journal of Plant Pathology 94: 243-252.
16. Jamaux I, Gelie B, Lamarque C (1995) Early stages of infection of rapeseed petals and leaves by Sclerotinia sclerotiorum revealed by scanning electron microscopy. Plant Pathology 44: 22-30.
17. Zheng AP, Wang YR (2011) The research of infection process and biological characteristics of Rhizoctonia solani AG-1 IB on soybean. Journal of Yeast and Fungal Research 2: 93-98.
18. Smith VL, Punja ZK, Jenkins SF (1986) A histological study of infection of host tissue by Sclerotium rolfsii. Phytopathology 76: 755-759.
19. Punja ZK, Grogan RG (1983) Germination and infection by basidiospores of Athelia (Sclerotium) rolfsii. Plant Disease 67: 875-878.
20. Baker CJ, Bateman DF (1978) Cutin degradation by plant pathogenic fungi. Phytopathology 68: 1577-1584.
21. Kaveriappa KM (1979) Mutual aversion in brinjal isolates of Sclerotium rolfsii. Indian Phytopathology 32: 475-477.
22. Cole AL, Bateman DF (1969) Arabanase production by Selerotium rolfsii and its role in tissue maceration. Phytopathology 59: 1750-1753.
23. Sadana JC, Shewale JG, Deshpande MV (1980) High Cellobiase and Xylanase Production by Sclerotium rolfsii UV-8 Mutant in Submerged Culture. Appl Environ Microbiol 39: 935-936.
24. Bateman DF (1972) The polygalacturonase complex produced by Sclerotium rolfsii. Physiological Plant Pathology 2: 175-184.

25. Felle HH, Herrmann A, Hanstein S, Hückelhoven R, Kogel KH (2004) Apoplastic ph signaling in barley leaves attacked by the powdery mildew fungus Blumeria graminis f. Sp. Hordei. Mol Plant Microbe Interact 17: 118-123.

26. Smith PH, Foster EM, Boyd LA, Brown JKM (1996) The early development of Erysiphe pisi on Pisum sativum L. Plant Pathology 45: 302-309.

27. Niks RE, Rubiales D (2002) Potentially durable resistance mechanisms in plants to specialized fungal pathogens. Euphytica 124: 216-216.

28. Wynn WK, Staples RC (1981) Tropism of fungi in host recognition. In: Staples RC, Toenilsen GA, eds. Plant disease control: resistance and susceptibility. New York: John Wiley 45-69.

29. Anker C, Niks RE (2001) Prehaustorial resistance to the wheat leaf rust fungus, Puccinia triticina, in Triticum monococcum (s.s). Euphytica 117: 209-215.

30. Hoch HC, Staples RC (1987) Structural and chemical changes among the rust fungi during appressorium development. Annual Review of Phytopathology 25: 231-247.

31. Rubiales D, Niks RE (1992) Low appressorium formation by rust fungi on Hordeum chilense leaves. Phytopathology 82: 1007-1012.

32. Vaz Patto MC, Fernandez-Aparicio M, Moral A, Rubiales D (2009) Pre- and post-haustorial resistance to rust in Lathyrus cicera L. Euphytica 165: 27-34.

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