Ascochyta mycoparasitica sp. nov., a Novel Mycoparasite of Sclerotium oryzae in California Rice Fields

Author(s): Richard D. Cartwright, Robert K. Webster and Carl M. Wick

Source: *Mycologia*, Vol. 89, No. 1 (Jan. - Feb., 1997), pp. 163-172
Published by: Mycological Society of America
Stable URL: http://www.jstor.org/stable/3761185
Accessed: 18-11-2015 20:21 UTC

Mycologia, 89(1), 1997, pp. 163–172.

Ascochyta mycoparasitica sp. nov., a novel mycoparasite of *Sclerotium oryzae* in California rice fields

Richard D. Cartwright[1]
Robert K. Webster

Department of Plant Pathology, University of California, Davis, California 95616

Carl M. Wick

UC Cooperative Extension, 2279 Del-Oro Ave., Suite B, Oroville, California 95965

Abstract: *Ascochyta mycoparasitica* sp. nov., a novel mycoparasite from sclerotia of the stem rot of rice fungus, *Sclerotium oryzae,* is described. It was distinguished from other *Ascochyta* spp. by its relatively thick-walled, papillate, black pycnidium (100–250 μm diam), guttulate, slightly obovoid conidia (13.5–17.6 × 5.7–6.8 μm), and fungicolous habit. *Ascochyta mycoparasitica* was isolated from field-collected sclerotia of *S. oryzae* at higher frequency in March than just after rice harvest (in September) and from fields employing either no residue management or conventionally tilled residue management. The fungus parasitized sclerotia of *S. oryzae* and *Rhizoctonia solani* AG 1-IB in culture but not of 6 other sclerotial fungi tested. *Ascochyta mycoparasitica* also attacked hyphae, spores and other structures of 16 of 42 fungal species tested in co-culture on cellophane strips coated with cornmeal agar, being most virulent on *S. oryzae, Harzia verrucosa,* and *Absidia glauca.*

Key Words: biocontrol, hyperparasitism, mycoparasitism, *Oryza sativa, Rhizoctonia solani,* systematics

INTRODUCTION

Stem rot caused by the fungus *Sclerotium oryzae* Catt. is the most important rice disease in California. It is monocyclic, with the long-lived sclerotia of *S. oryzae* providing primary inoculum for each crop in the continuous rice culture system of California (Tullis and Cralley, 1941; Krause and Webster, 1972). Disease control has been limited to burning of rice residue between crops to destroy the sclerotia. This practice is currently being curtailed by legislation due to air pollution and potential health risks (California State Legislature, 1991), and more desirable control methods are badly needed.

During investigations on biodecomposition of rice residue and biocontrol of stem rot in California, an unusual and novel mycoparasitic coelomycete was repeatedly isolated from sclerotia of the stem rot fungus collected from California rice fields (Cartwright, 1992). This new species was named *Ascochyta mycoparasitica* and is the subject of this report.

MATERIALS AND METHODS

Rice stubble (lower rice stems excluding roots) was collected after harvest in September and during December, January, February and March of each year of the study. Sclerotia of *S. oryzae* were extracted from stubble using a modification of the soil extraction method of Krause and Webster (1972) as follows. Stubble was chopped into 3–5 cm segments, blended in 500 mL distilled water (Waring blender) for 1 min, then washed onto a soil sieve stack (500/355/180 μm openings) and rinsed repeatedly for 2 min to collect dislodged sclerotia of *S. oryzae* on the 180 μm sieve. Sieve contents were washed into a plastic cup, 300 mL of water added, and allowed to stand overnight. Sclerotia floated to the surface and were vacuumed from the water surface using tubing attached to a small vacuum flask. The cup was rinsed, sclerotia in the vacuum flask returned, and distilled water added to 400 mL. While stirring, 4 mL commercial bleach (5.25% NaOCl) was added to surface disinfest the sclerotia. After stirring 3 min, contents were poured into a 250-mL vacuum filtration apparatus (Millipore Filter Corp.) containing Whatman No. 4 filter paper (7 cm diam). The bleach was drained off with weak vacuum leaving the sclerotia on the filter paper. Sclerotia on the sides of the unit were washed downward with a squeeze bottle containing 1% bleach, then rinsed 3 times with sterile distilled water. Sclerotia-laden filter paper was then placed in a sterile plastic 9-cm petri dish and air dried overnight in a transfer hood. The filtration/collection procedure was conducted inside a laminar flow transfer hood to prevent re-contamination of the sclerotia.

Surface disinfested sclerotia were sprinkled onto antibiotic-amended (30 μg/mL chlortetracycline and streptomycin sulfate) cornmeal agar (CMA) (CM103

Accepted for publication September 13, 1996.

[1] Current address: Cooperative Extension Service, P.O. Box 391, Little Rock, Arkansas 72203. Email: rcartwright@uaex.edu

Oxoid Ltd.) plates and incubated at room temperature (19 C night, 24 C day) under fluorescent lights (12 h photoperiod). Germination of sclerotia was noted by the production of conidia [*Nakataea sigmoidea* (Cavara)Hara state] or hyphae and sclerotia of *S. oryzae* and percent germination recorded after 7 d (Krause and Webster, 1972). Other fungi that grew out of the sclerotia were isolated on CMA and stored in water (Boesewinkel, 1976).

Isolates of *A. mycoparasitica* were induced to form pycnidia by the following method: 250 mL of V8 broth (Tuite, 1976) in a 500-mL Erlenmeyer flask was inoculated with a 6 mm diam CMA plug from the edge of a 7 d-old colony of the fungus and placed on a shaker for 7 d at 200 rpm and room temperature (25 C). The resulting culture was added to 750 mL of sterile 1% sodium alginate solution (Kelco MV, Kelgin, Inc.) in a sterile 1-L blender vessel (Waring). The mixture was blended 20 s, then portions were dripped with a pipette into sterile 0.25 M $CaCl_2$ solution to form calcium alginate pellets containing the fungus. After 1 min, pellets were removed to sterile plastic petri dishes and air-dried overnight in a laminar flow transfer hood. Air-dried pellets were placed on 1% water agar plates and incubated under fluorescent lights (25–35 cm above plates) 5–14 d to allow pycnidia to form on the pellet surface. Pycnidia were excised from pellets and crushed or sectioned with a freezing microtome for compound light microscope study. Distilled water or lactophenol/cotton blue were generally used as mountants with measurements made accordingly. The following light microscopy types were routinely used during this study: bright field (BF), differential interference contrast (DIC) phase contrast (PC). For scanning electron microscopy (SEM), pycnidia were fixed, sectioned and observed as described later in this section for sclerotia. Colony characteristics were noted after 7 d at 25 C under fluorescent lights (12 h photoperiod) on CMA and PDA.

Living cultures are maintained by the senior author and a representative strain has been deposited in the International Mycological Institute.

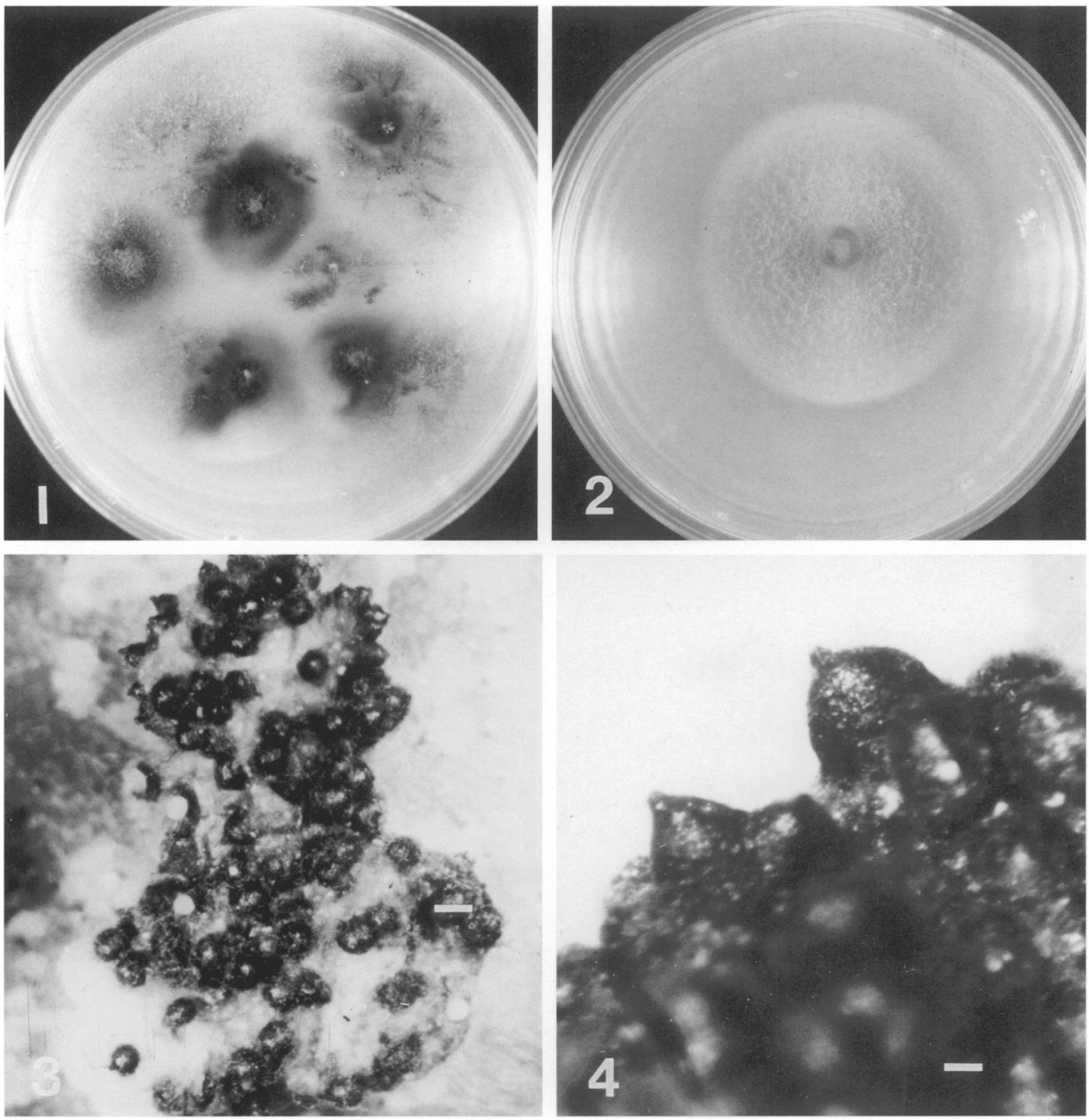
Sclerotial parasitism by *A. mycoparasitica* was tested using sclerotia of *S. oryzae* and other sclerotial fungi produced in the laboratory. Sclerotia of *S. oryzae* were produced using the method of Krause and Webster (1972) and of other test fungi by growing on potato dextrose agar (PDA, Oxoid, Ltd.) plates and harvesting after 3 wk. Sclerotia of test host fungi (20 per plate except *S. oryzae* ~100 per plate) were aseptically placed at the center of a CMA plate. An agar (CMA) plug (6 mm diam) from the edge of a 10-d-old colony of *A. mycoparasitica* was placed on the sclerotia and the co-culture was incubated at 10 C for 60 d. After 60 d, sclerotia were harvested, surface disinfested for 3 min in 1% bleach as before, air-dried and placed on CMA plates and incubated under lights for 7–14 d. Test sclerotia were then evaluated for germination and growth, as well as the presence of *A. mycoparasitica*. This experiment was repeated to verify results.

After visual evaluation, test sclerotia were mounted in 5% carboxymethyl-cellulose (CMC, Sigma), coarsely sectioned (20–40 μm) on a freezing microtome and inspected for internal colonization using light microscopy. For more detailed observation, sclerotia were also fixed in phosphate buffered (pH 7) 2% glutaraldehyde overnight, rinsed twice with distilled water, mounted in CMC, and sectioned by the freezing microtome as before. Sections were post fixed in buffered 1% osmium tetroxide for two h, and dehydrated with ethanol in the following series: 10, 20, 30, 40, 50, 60, 75, 95, 95, 100, 100, 100% using 15 min per step except for 30 min per 100% step. After dehydration, sections were critical-point dried using CO_2 as the carrier gas. Dried sections were mounted on stubs with double stick tape, sputter coated with 20–30 nm gold, and viewed at 10 KV in an International Scientific Instruments scanning electron microscope.

For fungal host range determination of *A. mycoparasitica,* co-cultures with test fungi were made by inoculating CMA coated 5 × 1.5 cm strips (2 per plate) of sterile cellophane placed on the surface of 1% water agar (L103 Oxoid Ltd.) plates with agar plugs (6 mm) from the edge of 7–14 d-old colonies (CMA) of each fungus. Plugs were placed about 2 cm apart on the strips and incubated under lights at room temperature for 2–4 wk to allow the fungi to grow together. Sections of cellophane containing the interaction region of the two fungi were aseptically excised and placed in lactophenol cotton blue on a glass slide. A cover slip was carefully added and the specimen examined with a compound microscope. Observations of mycoparasitism were noted photographically and by the following rating scale: 0 = no parasitism, 1 = parasitism observed, slow and restricted development, 2 = parasitism observed, moderate development within host, 3 = extensive parasitism observed, widespread and aggressive development within host. Co-cultures of all combinations were repeated at least 3 times to verify observations. In some cases, cellophane sections bearing co-cultures of interest were processed for scanning electron microscopy as before (no freezing microtome step).

RESULTS

Ascochyta mycoparasitica Cartwright et Webster, sp. nov. FIGS. 1–24

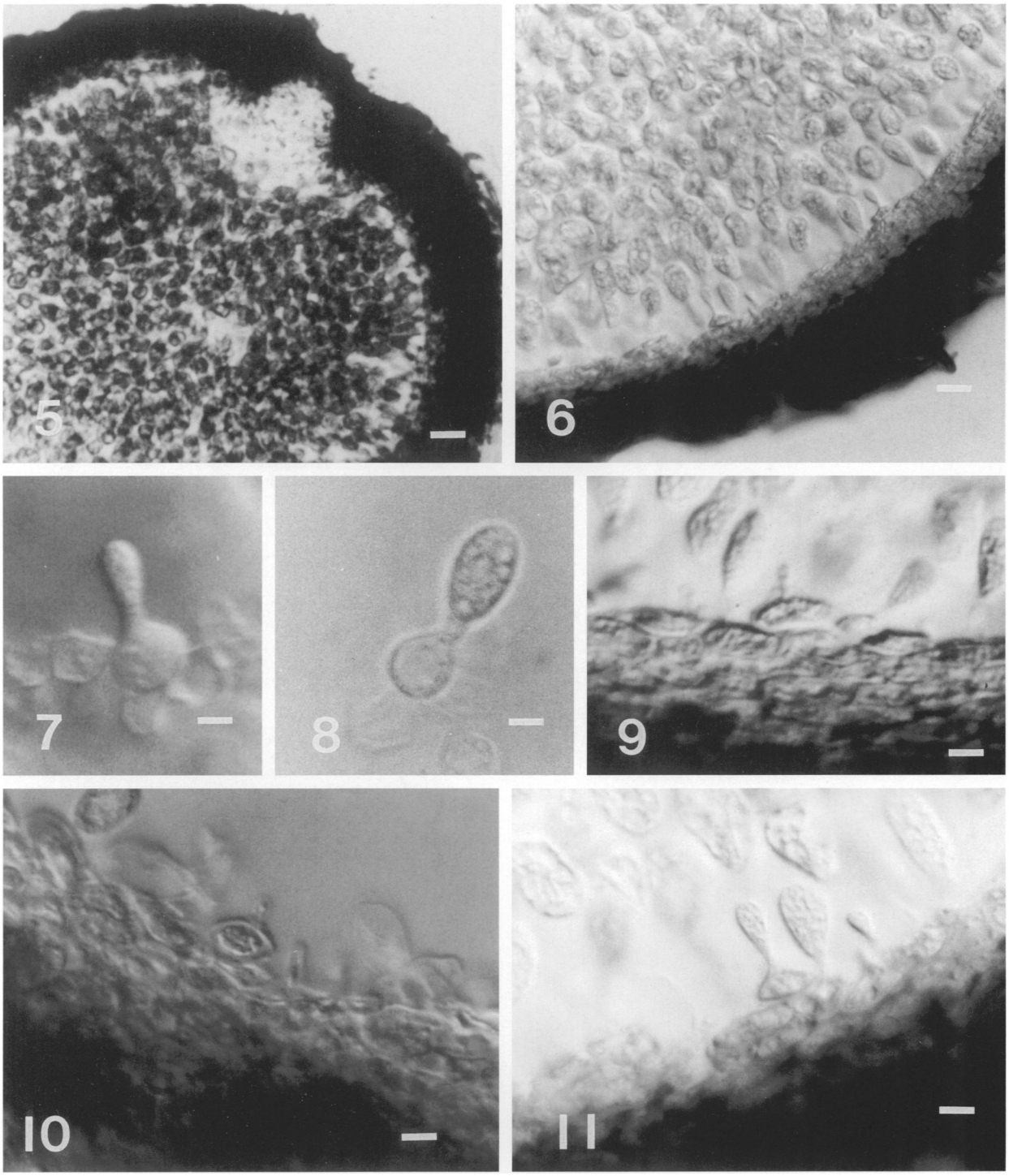


FIGS. 1–4. *Ascochyta mycoparasitica.* 1. Colonies on CMA growing from surface disinfested sclerotia of *Sclerotium oryzae.* 2. Colony (7 d) on CMA. 3, 4. Pycnidia on calcium alginate pellets. Scale bars: FIG. 3 = 400 μm, 4 = 75 μm.

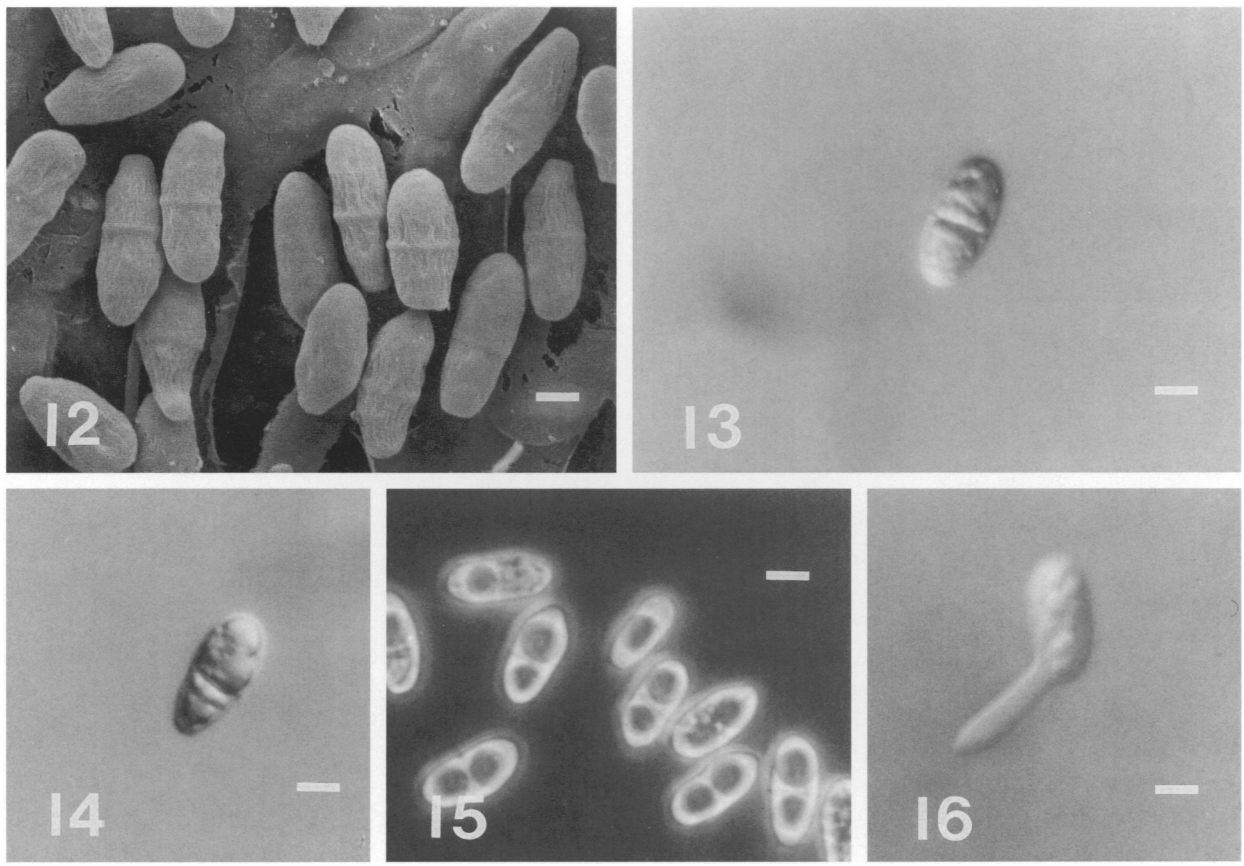
Pycnidia atra, globosa, plerumque superficialia, glabra vel modice pilosa, singula vel aggregata, 100–250 μm diametro, parietes ex textura angulari, 5–12 cellulis crassi, ostiolum centrale, circulare, papillatum, 20–40 μm diametrum. Cellulae conidiogenae (temporariae) hyalinae, simplices, holoblasticae, ampulliformes, 5.4–7.7 × 4.8–8.1 μm, exoriens pariete interiori pycnidii, separatus facile, saepe exsudans cum conidia; (permanentes) hyalinae, simplices, phialidicae, ampulliformes, 6.0–8.5 × 2.6–7.2 μm, colla variabilium longitudum (<1–6 μm), exoriens pariete interiori pycnidii. Conidia hyalina, tenuiter tunicata, levigata, ellipsoidea vel obovoidea, apex obtusus, fundus saepe truncatus, mediane vel nonaequale uniseptata ubi matura, guttulata, cellulae uninucleatae, 13.5–17.6 × 5.7–6.8 μm.

HOLOTYPUS. In calcii alginati culturo e sclerotio (*Sclerotium oryzae*), Butte Co., California, U. S. A., 14 Jan 1990, *R. D. Cartwright* (BPI).

Pycnidia black, globose, mostly superficial, glabrous to somewhat hairy, solitary to aggregated, 100–250 μm diam, walls textura angularis, 5–12 cells thick, ostiole central, circular, papillate, 20–40 μm diam. Two types of *conidiogenous cells* formed (FIGS. 7–11; *sensu* Punithalingam, 1979). Temporary cells hyaline, holoblastic, ampulliform, 5.4–7.7 × 4.8–8.1 μm, arising from inner pycnidial wall, easily de-



FIGS. 5–11. *Ascochyta mycoparasitica*. 5. Section through ostiole of pycnidium mounted in lactophenol cotton blue (BF). 6. Section of pycnidium mounted in water showing inner and outer walls (DIC). 7, 8. Temporary holoblastic conidiogenous cells with developing conidia mounted in water [DIC (7) and BF (8)]. 9–11. Permanent phialides on inner wall of pycnidium mounted in water (DIC). Scale bars: FIG. 5 = 20 μm, 6 = 15 μm, 7–11 = 4 μm.



FIGS. 12–16. *Ascochyta mycoparasitica* conidia. 12. Mature conidia (SEM). 13, 14. Mature guttulate conidium mounted in water (DIC). 15. Mature conidia mounted in water (PC). 16. Germinating conidium mounted in water (DIC). Scale bars: FIG. 12 = 4.5 μm, 13, 14 = 5 μm, 15 = 8 μm, 16 = 5 μm.

tached, often exuded with spores; permanent cells hyaline, phialidic, ampulliform, 6.0–8.5 × 2.6–7.2 μm, necks short to long (<1–6 μm), arising from inner pycnidial wall. *Conidia* hyaline, thin-walled, smooth, ellipsoid to obovoid, apex obtuse, base often truncate, medianly to unequally uniseptate at maturity, guttulate, cells uninucleate, 13.5–17.6 × 5.7–6.8 μm. [The description given above was from pycnidia produced on calcium alginate pellets incubated on water agar under fluorescent lights (12 h) at room temperature (25 C)].

Colonies on CMA 2.9–3.5 cm diam, gray and often partially reddish-pink to reddish-brown when grown with other fungi; reddish ring sometimes forming around the center of older colonies. On PDA, colonies 2.7–3.2 cm diam, gray with an off-white even border and wooly aerial mycelium; reverse of colony black. Pycnidia forming rarely and sparsely—if at all—on CMA and never on PDA; forming in abundance within 6 d on calcium alginate pellets incubated on water agar under fluorescent lights; occasionally forming on inoculated sterile rice stems after 30 d at room temperature (25 C) under fluorescent lights (12 h) and were similar to those formed on calcium alginate, however, they tended to be subglobose and immersed.

Habitat and distribution. On sclerotia of *Sclerotium oryzae* and *R. solani* Kühn in rice fields. Arkansas and California.

HOLOTYPE. UNITED STATES. CALIFORNIA: Butte County, dry calcium alginate culture from sclerotia of *Sclerotium oryzae*, 14 Jan 1990, *R. D. Cartwright* (BPI).

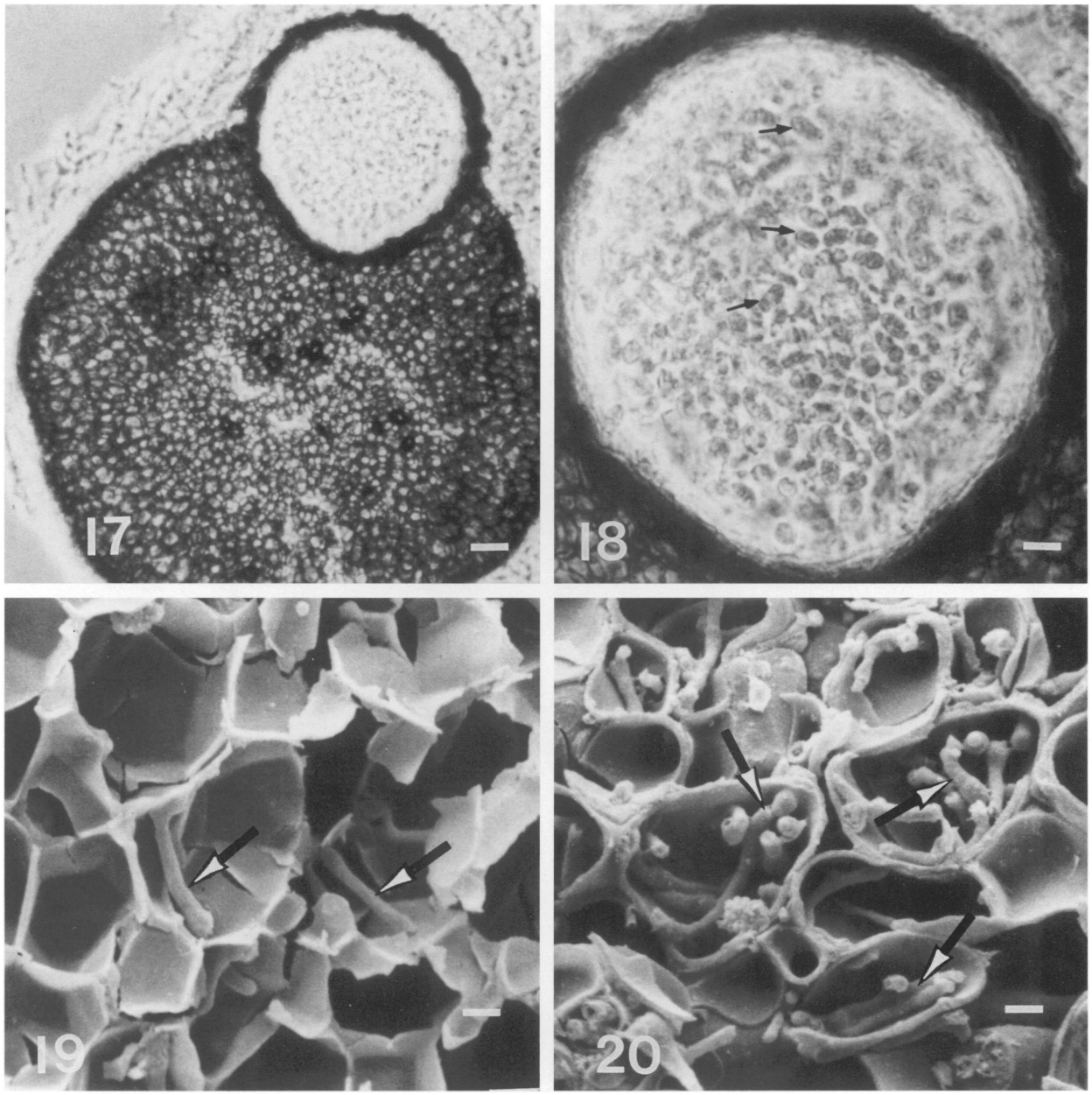
Strains examined. From sclerotia of *S. oryzae*: UNITED STATES. ARKANSAS: Lawrence County, 4 strains fruiting on calcium alginate pellets, 1992, *R.D. Cartwright AC1-4*. CALIFORNIA: Butte County, 11 strains fruiting on calcium alginate pellets, 1990–1991, *R.D. Cartwright C1-C11*. Isolate C3 on calcium alginate pellets, 14 Jan.1990, *R.D. Cartwright C3* (IMI 349994).

From sclerotia of *R. solani* AG 1-IA: UNITED STATES. ARKANSAS: Lawrence County, 2 strains fruiting on calcium alginate pellets, 1992–1993, *R.D. Cartwright AC1RS-AC2RS*.

These are dry cultures and are maintained in the personal herbarium of the senior author.

Ascochyta mycoparasitica was isolated at greater frequency from stubble-borne sclerotia collected later in the winter compared to those collected earlier (TABLE I). Sclerotia from fields that had been burned or flooded during the winter months yielded the mycoparasite at a much lower level than sclerotia from fields where the residue was untreated or managed using conventional cultivation practices (TABLE I).

Of the sclerotial fungi tested in the laboratory, only *S. oryzae* sclerotia were effectively colonized (100%) and in-

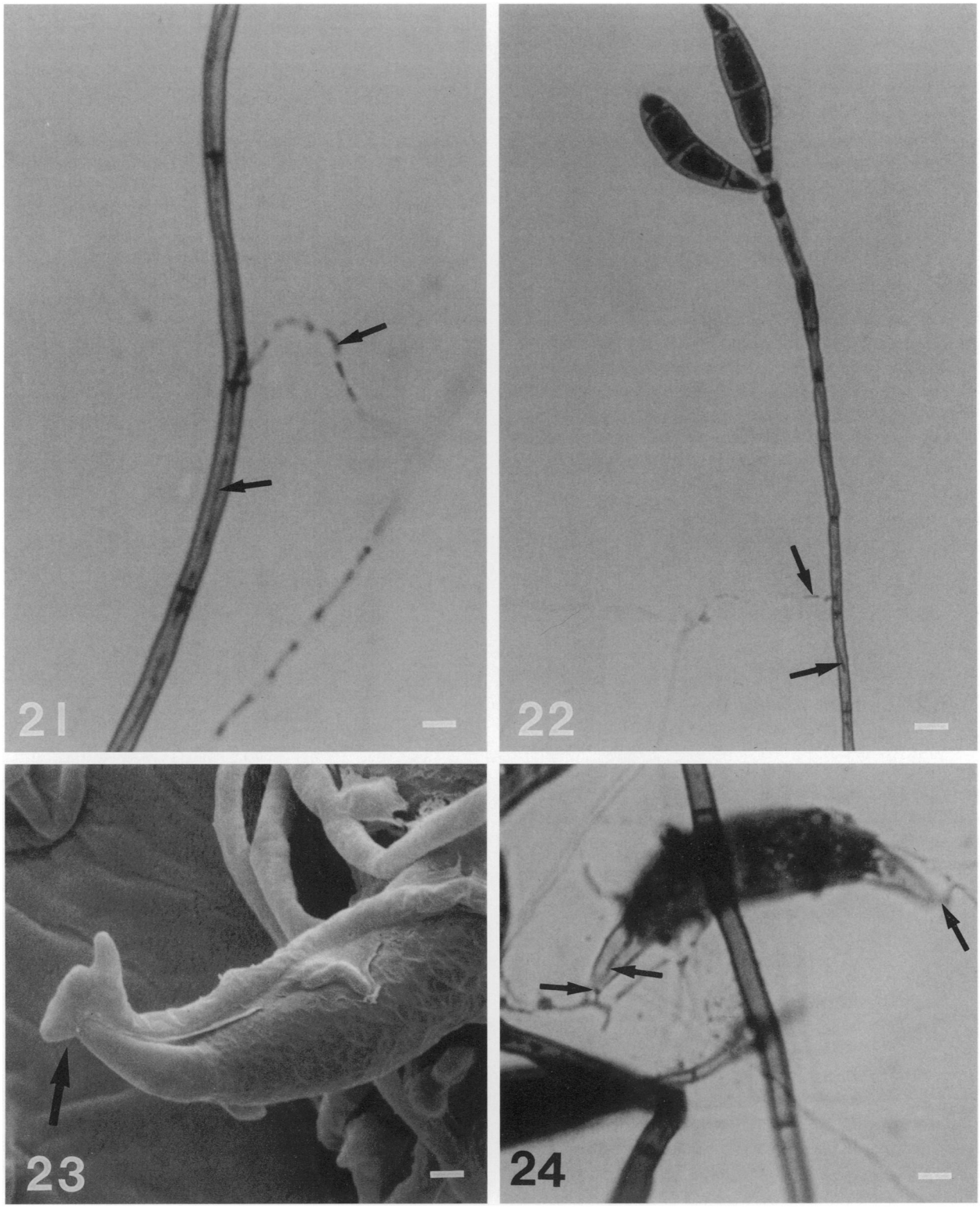


FIGS. 17–20. *Ascochyta mycoparasitica* parasitizing sclerotia of *Sclerotium oryzae* and *Rhizoctonia solani* AG 1-IB in culture. 17, 18. Section of pycnidium of *A. mycoparasitica* partially within sclerotium of *S. oryzae,* mounted in lactophenol cotton blue (PC); arrows in 18 indicate conidia. 19. Hyphae of *A. mycoparasitica* (arrows) inside cells of *S. oryzae* sclerotium (SEM). 20. Hyphae of *A. mycoparasitica* (arrows) inside cells of *R. solani* AG 1-IB sclerotium (SEM). Scale bars: FIG. 17 = 18 μm, 18 = 7 μm, 19 = 4 μm, 20 = 5 μm.

hibited by *A. mycoparasitica* with a reduction in germination from 83 to 13% for uninoculated vs inoculated treatments (TABLE II). *Ascochyta mycoparasitica* was reisolated at various levels from surface disinfested sclerotia of *Rhizoctonia oryzae-sativae* (Sawada) Mordue, *Sclerotium hydrophilum* Sacc., *Rhizoctonia solani* AG 1-IA and IB, but did not inhibit their germination (TABLE II).

Sclerotial sections viewed by scanning electron microscopy revealed internal colonization of sclerotia of *S. oryzae* and *R. solani* AG 1-IB by *A. mycoparasitica.* Sclerotia of other test fungi showed no penetration or internal colonization by the parasite. Rarely, *A. mycoparasitica* formed pycnidia partially within infected sclerotia of *S. oryzae.*

When co-cultured on CMA coated cellophane with other fungi, *A. mycoparasitica* parasitized the hyphae of several fungi (TABLE III). It was rated most virulent on *S. oryzae, Absidia glauca* Hagem, and *Harzia verrucosa* (Togn.) Hol.-Jech. and less so on *Ascochyta pisi* Lib., *Mucor* spp., *Rhizoctonia* spp., *Sclerotium hydrophilum* Sacc., *Waitea circinata* Warcup & Talbot, *Gelasinospora retispora* Cain, *Mortierella*



FIGS. 21–24. *Ascochyta mycoparasitica* parasitizing *Sclerotium oryzae* in culture. 21. Hypha of *A. mycoparasitica* (arrows) within hypha of *S. oryzae,* mounted in lactophenol cotton (BF). 22. Hypha of *A. mycoparasitica* (arrows) within conidiophore of *S. oryzae* [=*Nakataea sigmoidea*], mounted in lactophenol cotton blue (BF). 23. *Ascochyta mycoparasitica* (arrow) attacking germ pore of *S. oryzae* [=*N. sigmoidea*] conidium (SEM). 24. Hyphae of *A. mycoparasitica* attacking conidium of *S. oryzae* [=*N. sigmoidea*] via germ pores (arrows), mounted in lactophenol cotton blue (BF). Scale bars: FIG. 21 = 8 μm, 22 = 10 μm, 23 = 2.5 μm, 24 = 10 μm.

TABLE I. Isolation of *A. mycoparasitica* from surface disinfested sclerotia of *S. oryzae* from rice stubble from different California rice fields during the fall and winter of 1989–1991

Month collected	Frequency[a]	Residue management[b]	Frequency[c]
September	0.0	fall disk/winter flood	0.6
December	1.1	winter flood/rolled	1.2
January	4.2	fall burn	1.2
February	6.2	fall rolled	4.2
March	6.6	none	4.3
		fall disked	6.3
		fall rolled & chiseled	9.8
MSD.05[d]	3.8		6.8

[a] Percentage of surface disinfested sclerotia of *S. oryzae* collected from rice stubble at different times from which *A. mycoparasitica* was isolated.

[b] Cultural practices employed to eliminate rice residue left in the field after harvest.

[c] Percentage of surface disinfested sclerotia of *S. oryzae* collected in March from rice stubble under different residue management systems from which *A. mycoparasitica* was isolated.

[d] MSD = Minimum Significant Difference according to Tukey's HSD test, P = 0.05.

TABLE II. Colonization and parasitism of sclerotial fungi by *A. mycoparasitica* in culture

Test fungus	Uninoculated	Inoculated	
	Germinated[a]	Germinated[a]	Colonized[b]
Botrytis cinerea[c]	100	100	0
Rhizoctonia oryzae-sativae	100	99	35
Rhizoctonia solani AG 1-IA	100	100	1
Rhizoctonia solani AG 1-IB[d]	100	100	73
Sclerotinia minor[c]	100	100	0
Sclerotium hydrophilum	99	100	61
Sclerotium rolfsii[c]	100	100	0
Sclerotium oryzae[d]	83	13	100

[a] Percent of sclerotia that germinated after 2 mo incubation at 10 C either uninoculated or inoculated with *A. mycoparasitica*. After 2 mo, sclerotia were surface disinfested with 1% bleach for 3 min and plated on CMA at room temperature.

[b] Percent of sclerotia yielding *A. mycoparasitica* after incubation and surface disinfestation as described above.

[c] *Botrytis cinerea* Pers.:Fr.; *Sclerotinia minor* Jagger; *Sclerotium rolfsii* Sacc.

[d] Internal colonization of infected sclerotia by *A. mycoparasitica* observed.

elongata Linnem., and *Trichoderma harzianum* Rifai (TABLE III). *Ascochyta mycoparasitica* did not parasitize the remaining 26 fungi tested (*Acremonium* sp., *A. strictum* W. Gams, *A. terricola* (Miller et al.) W. Gams, *Alternaria alternata* (Fr.: Fr.) Keissler, *Aspergillus terreus* Thom, *Ceratocystis* sp., *Chrysosporium* sp., *Cladosporium cladosporioides* (Fresen) G. A. de Vries, *Coprinus* sp., *Epicoccum nigrum* Link, *Fusarium culmorum* (W. G. Smith) Sacc., *F. graminearum* Schwabe, *F. reticulatum* Mont., *F. avenaceum* (Fr.:Fr.) Sacc., *F. moniliforme* J. Sheld., *Gelasinospora seminuda* Cailleux, *Humicola grisea* Traaen var. *grisea*, *Microdochium bolleyi* (Sprague) de Hoog & Hermanides-Nijof, *Neurospora* sp., *Papulaspora irregularis* Hotson, *Penicillium* sp., *Phoma leveillei* Boerema & Bollen, *Phoma medicaginus* Malbr. & Roum. var. *pinodella* (L. K. Jones) Boerema, *Trichoderma viride* Pers.:Fr., and *Ulocladium atrum* Preuss).

TABLE III. Host range of *A. mycoparasitica* in culture

Test fungus	Rating[a]	Host structures attacked
Sclerotium oryzae	3	hyphae, conidia, sclerotia
Absidia glauca	3	hyphae, columella, sporangia
Harzia verrucosa	3	hyphae, conidia, conidiophores
Ascochyta pisi	2	hyphae
Mucor hiemalis	2	hyphae, columella, sporangia
M. mucedo	2	hyphae, columella, sporangia
Rhizoctonia (binucleate)	2	hyphae
R. oryzae-sativae	2	hyphae
R. solani AG 1-IA	2	hyphae
R. solani AG 1-IB	2	hyphae, sclerotia
R. solani AG 4	2	hyphae
Sclerotium hydrophilum	2	hyphae
Waitea circinata	2	hyphae
Gelasinospora retispora	1	ascospores
Mortierella elongata	1	hyphae
Trichoderma harzianum	1	hyphae

[a] Rating system: 0 = no parasitism; 1 = parasitism, slow and restricted development; 2 = parasitism, moderate development; 3 = parasitism, rapid and aggressive colonization.

DISCUSSION

Ascochyta mycoparasitica was placed in *Ascochyta* Lib. based on pycnidial and conidial morphology and conidiogenesis as specified in the revised description of the genus by Punithalingam (1979, 1988). Our interpretation of conidiogenesis and conidiation in *A. mycoparasitica* in culture was very similar to that described by Punithalingam (1979) for *Ascochyta*. Within young pycnidia, conidia were produced from temporary (sensu Punithalingam, 1979) conidiogenous cells that separated readily from the inner pycnidial wall and into the central cavity. When young pycnidia

were squashed or placed in water on a microscope slide, these cells with attached conidia were exuded with the conidial mass (FIGS. 7, 8). As the pycnidia matured, conidia were formed predominately from what appeared to be permanent (sensu Punithalingam, 1979) conidiogenous cells that were part of the inner pycnidial wall and which would not separate easily (FIGS. 6, 10, 11). We could find no other reference to this type of conidiation in the genus *Phoma* Sacc., which includes a few species that form a percentage of septate conidia. According to Punithalingam, some authorities believe the permanent conidiogenous cells in *Ascochyta* to be annellidic, but this is difficult to interpret in many species, especially with light microscopy. We did not clearly observe annelides in either *A. mycoparasitica* or an available *A. pisi* isolate. In *A. mycoparasitica,* we usually observed septation of the conidium either late in development and while still attached to the conidiogenous cell or shortly after separation from the conidiogenous cell. This latter interpretation was based on the high percentage of septate conidia (>90% uniseptate) within the pycnidial cavity only a few days after pycnidial formation. In *Phoma,* septation takes place completely independently of conidogenesis and in most species involves less than 10% of the conidia. We could not determine the nature of septation in *A. mycoparasitica,* which was mentioned by Punithalingam as a character emphasized by Boerema and Bollen (1975). We observed squashed and sectioned conidia of both *A. mycoparasitica* and *A. pisi* and were unable to see any consistent evidence of distoseptation. We therefore did not feel justified in using this character in distinguishing the placement of *A. mycoparasitica* in either *Ascochyta* or *Phoma* and agreed with Punithalingam (1979) on the lack of usefulness of this character in some *Ascochyta* species. While the broad generic interpretation of *Ascochyta* by Punithalingam (1975) requires inclusion of *A. mycoparasitica,* several features of this new species—including the thick-walled, black pycnidium, the often near obovoid conidia, and the fungicolous habit—are not commonly observed in this form-genus.

Because it was isolated from rice fields, comparison with descriptions of graminicolous *Ascochyta* spp. (Punithalingam, 1979, 1988) similar to *A. mycoparasitica* in conidial shape and size, viz *A. imperatae* Punith., *A. graminea* (Sacc.) R. Sprague & A.G. Johnson, and *A. oryzina* Hara were made. Differences in pycnidial size, shape, color and conidial and conidiogenous cell shape differentiated *A. mycoparasitica* from these species. As described, *A. oryzina* and *A. graminea* were closest to *A. mycoparasitica,* but differed in having narrower conidia (only 4–5 μm wide). Both species were described as having brown or rust-brown pycnidia with undetermined wall thickness while the pycnidia of *A. mycoparasitica* were black and thick-walled.

Because *A. mycoparasitica* is fungicolous, it may have closer biological affinity with *Sphaerellopsis* Cke., once included in *Ascochyta* Lib. based on pycnidial and conidial morphology. *Sphaerellopsis filum* (Biv.-Bern. ex Fr.) Sutton [= *Darluca filum* (Biv.-Bern. ex Fr.) Berk.], is a well known parasite of rust fungi (Sutton, 1980), has a thick-walled black pycnidium and uniseptate conidia similar in size and shape to *A. mycoparasitica.* Unlike *A. mycoparasitica* however, its pycnidia are eustromatic, conidia tend to turn light brown with age, often have a gelatinous cap, and conidiogenous cells apparently have annellides. Reports of mycoparasitic coelomycetes in the literature are rare.

Ascochyta mycoparasitica effectively infected and colonized sclerotia of only *S. oryzae* and *Rhizoctonia solani* AG 1-IB, indicating a degree of host specialization. While infected sclerotia of *S. oryzae* seldom germinated, those of *R. solani* AG 1-IB germinated readily but subsequent hyphal growth was reduced. This result was unexpected as the degree of internal colonization by the mycoparasite was greater in sclerotia of the latter fungus. Although recovered from a low percentage of inoculated then surface disinfested sclerotia of *Rhizoctonia solani* AG 1-IA, *A. mycoparasitica* did not infect sclerotia of this fungus in these tests as revealed by microscopic inspection of sclerotial sections. Though consistent over two experiments, this result is puzzling because *R. solani* AG 1-IA is similar to *R. solani* AG 1-IB, and because *A. mycoparasitica* was recently isolated by the first author from field collected sclerotia of *R. solani* AG 1-IA from Arkansas rice fields.

When co-cultured on cellophane strips with other fungi, *A. mycoparasitica* parasitized a limited number of unrelated species. It vigorously attacked the conidia of *Sclerotium oryzae* [=*Nakataea sigmoidea*] and *Harzia verrucosa. Ascochyta mycoparasitica* also parasitized *Absidia glauca, Mucor hiemalis* Wehmer, and *Mucor mucedo* Mich. ex St.-Am. demonstrating a host range outside the higher fungi. Interestingly enough, *A. mycoparasitica* infected some structures by what seemed to be site-specific penetration, such as through the germ pore of *Nakataea* conidia (FIGS. 23,24). When inside hyphae of a susceptible host, *A. mycoparasitica* grew relatively unrestricted from cell to cell, destroying contents along the way (FIGS. 21,22). Coiling about host hyphae as reported for mycoparasitic *Pythium* spp. and *Trichoderma* spp. (Baker, 1987) was rarely observed with *A. mycoparasitica.*

Rhizoctonia spp., while penetrated by *A. mycoparasitica,* resisted intrahyphal colonization and the na-

ture of this resistance remains unknown. *Trichoderma harzianum,* another mycoparasite, was attacked by *A. mycoparasitica* but hyphae of the latter was often damaged or destroyed in the interaction zone of the two. Since *T. harzianum* produces copious amounts of chitinolytic enzymes in culture, this was hypothesized as the cause of the observed damage to *A. mycoparasitica.* It was noted that *T. harzianum* formed chlamydospores in the interaction zone of the two fungi.

Biocontrol of sclerotial plant pathogens using indigenous mycoparasites remains largely theoretical, with most major contributions in the area derived from studies on *Sclerotinia sclerotiorum* (Lib.) de Bary (Trutmann, et al., 1980; Ghaffar, 1988; Whipps and Budge, 1990). Efforts on biocontrol of stem rot and other sclerotial diseases of rice are indeed rare (Usmani and Ghaffar, 1974; Cartwright, 1992) and it remains a hope that the difficult research to replace or improve ineffective or environmentally damaging control methods for these diseases will proceed more vigorously in the future.

ACKNOWLEDGMENTS

The authors thank Dr. E. E. Butler and Dr. P. S. Gunnell for cultures of various fungi used in this study and for helpful advice. We also thank Dr. J. Adaskaveg for assistance with light and SEM microscopy and comments. Special thanks go to Drs. C. D. Boyette, W. Connick, and G. E. Templeton for advice on incorporation of fungi in calcium alginate. We thank Dr. M. Brandon and the personnel of the California Cooperative Rice Foundation Experiment Station near Biggs for assistance and encouragement during the field collection phase of this work. We offer sincere thanks to the California Rice Research Board for partial funding of this research and to the many rice producers that allowed us access to their fields during the course of this study. A very special thanks is extended to Dr. Daniel B. Levine, Dept. of Foreign Languages, University of Arkansas for invaluable assistance with the Latin description.

LITERATURE CITED

Baker, R. 1987. Mycoparasitism: ecology and physiology. *Canad. J. Pl. Pathol.* 9: 370–379.

Boerema, G. H. and, G. J. Bollen. 1975. Conidiogenesis and conidial septation as differentiating criteria between *Phoma* and *Ascochyta. Persoonia* 8: 111–144.

Boesewinkel, H. J. 1976. Storage of fungal cultures in water. *Trans. Brit. Mycol. Soc.* 66: 183.

California State Legislature. 1991. AB 1378—The Rice Straw Burning Reduction Act of 1991.

Cartwright, R. D. 1992. *Biodecomposition of rice residue and biocontrol of* S. oryzae *in California.* Ph.D. Thesis, UC Davis, Davis. 161 pp.

Ghaffar, A. 1988. Biological control of sclerotial diseases. Pp. 154–169. *In: Biocontrol of plant diseases,* Vol. I. Eds., K. G. Mukerji and K. L Garg. CRC Press, Boca Raton, Florida.

Krause, R. A., and R. K. Webster. 1972. Sclerotial production, viability determination and quantitative recovery of *Sclerotium oryzae* from soil. *Mycologia* 64: 1333–1337.

Punithalingam, E. 1979. *Graminicolous Ascochyta species. Mycol. Pap.* 142: 1–214.

———. 1988. *Ascochyta* II. Species on monocotyledons (excluding grasses), cryptogams and gymnosperms. *Mycol. Pap.* 159: 1–235.

Sutton, B.C. 1980. *The Coelomycetes.* Commonwealth Mycological Institute, Kew, United Kingdom. 696 pp.

Trutmann, P., P. J. Keane, and P. R. Merriman. 1980. Reduction of sclerotial inoculum of *Sclerotinia sclerotiorum* with *Coniothyrium minitans. Soil Biol. Biochem.* 12: 461–465.

Tuite, J. 1976. *Plant pathological methods: fungi and bacteria.* Burgess Publishing Co., Minneapolis. 239 pp.

Tullis, E.C. and E.M. Cralley. 1941. Longevity of sclerotia of the stem-rot fungus *Leptosphaeria salvinii. Phytopathology* 31: 279–281.

Usmani, S. M. H., and A. Ghaffar. 1974. Biological control of *Sclerotium oryzae* Catt., the cause of stem rot of rice. 1. Population and viability of sclerotia in soil. *Pakistan J. Bot.* 6: 157–162.

Whipps, J. M., and S. P. Budge. 1990. Screening for sclerotial mycoparasites of *Sclerotinia sclerotiorum. Mycol. Res.* 94: 607–612.