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Rhexocercosporidium panacis sp. nov., a new anamorphic species causing rusted root of ginseng (*Panax quinquefolius*)

R.D. Reeleder[1]

Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, Ontario, N5V 4T3 Canada

Abstract: A new species of the anamorphic genus *Rhexocercosporidium* is described. Isolates of a *Rhex-Rhexocercosporidium* sp. were obtained from ginseng (*Panax quinquefolius*) roots with symptoms of rusted root. These isolates were found to be genetically and morphologically distinct from the only described species in this genus, *R. carotae*. Sequence data from the ribosomal DNA region spanning the internal transcribed spacers 1 and 2 and from a portion of the β-tubulin gene of the ginseng *Rhexocercosporidium* were compared to those of *R. carotae*. Parsimony analyses of sequence data showed that *R. carotae* and the ginseng isolates belonged to distinct but closely related clades. Conidia of a typical ginseng isolate were significantly shorter and possessed fewer septa than *R. carotae* but shared rhexolytic secession of conidia with *R. carotae*. The binomial *Rhexocercosporidium panacis* is proposed to accommodate isolates of this genus that are associated with the rusted root disease.

Key words: *Acrothecium*, β-tubulin, ginseng, ITS, Leotiomycetes, molecular phylogenetics, *Panax*, *Pseudocercosporidium*, rusty root

INTRODUCTION

Rusted root (also known as rusty root) is a serious disease of ginseng (*Panax quinquefolius* L.) in North America. Although first described in the 1930s (Hildebrand 1935), the cause of the disease was determined only recently (Reeleder and Hoke 2005, Reeleder et al 2006) and reported to be a member of the genus *Rhexocercosporidium*, an anamorphic genus related to the Leotiomycetes (Shoemaker et al 2002). Evidence is provided herein that the ginseng isolates of this fungus are distinct from *R. carotae* (Årsvoll) U. Braun, the only species currently described for the genus, and it is concluded that the ginseng isolates represent a new species, *Rhexocercosporidium panacis* sp. nov.

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[1] E-mail: reelederr@agr.gc.ca Telephone: (519) 457-1470 x297

MATERIALS AND METHODS

Collection.—Cultures of *Rhexocercosporidium* were obtained from ginseng roots with symptoms of rusted root. Roots with characteristic disease symptoms (Hildebrand 1935, Reeleder et al 2006) were obtained from cooperating growers in the provinces of British Columbia and Ontario, Canada. Roots were washed with tap water and cut crosswise to produce sections of diseased tissue approx 1 cm long. Root pieces were disinfested with 1% sodium hypochlorite for 1 min and washed twice with sterile water. Five by five mm sections of disinfested tissue were placed on MRBA medium (Reeleder et al 2002). Inoculated plates of MRBA were kept in darkness at room temperature (22 ± 2 C) for 2 d then exposed to ambient light an additional 5–20 d. A slow-growing fungus with olivaceous gray (Rayner 1970) mycelium commonly was observed growing from diseased tissue. Representatives were transferred to V8 agar (Tuite 1969). Preliminary morphological examination and sequence data (see below) indicated that the fungus belonged to the anamorphic genus *Rhexocercosporidium*. Single-spore isolates of 10 cultures (six obtained from infected roots grown in British Columbia, and four from Ontario-grown infected roots) were stored on clarified V8 agar slants at room temperature (20 ± 2 C) and in 15% glycerol at −80 C until required.

Cultures of *R. carotae* were obtained from the Canadian Collection of Fungal Cultures (Ottawa, Ontario) and from Dr J. Köhl, Plant Research International, Wageningen, The Netherlands. Additional fungal cultures were obtained from Dr L. Wick, UFZ Centre for Environmental Research, Leipzig, Germany; Dr C. Grau, University of Wisconsin, Madison (WI); and Dr A. Osbourn, Sainsbury Laboratory, Norwich, UK. Culture information is provided (TABLE I).

Colony morphology and color.—Selected cultures of the ginseng *Rhexocercosporidium* and *R. carotae* were compared with respect to colony morphology on clarified V8 broth (Tuite 1969), V8 agar and MRBA. Glass Petri dishes containing 15 mL of V8 broth and disposable plastic Petri dishes containing 15 mL of V8 agar were inoculated with 5 mm agar plugs from 2–3 wk old cultures then incubated at 18 ± 1 C with a 12 h photoperiod for 3 wk. Resulting colonies were compared by noting colony morphology. Color of colonies was determined with a mycological color chart (Rayner 1970).

Conidia and conidiogenous cells.—Conidia from 14–21 d old V8 broth cultures were examined at 400×; length and width of 100 conidia each of ginseng *Rhexocercosporidium* isolate RRD1 and *R. carotae* DAOM 226960 were determined with a Zeiss Axioskop microscope equipped for phase contrast microscopy. Sixty conidia of the sparsely sporulating isolate DSE48.1b were examined (1000×). Images of conidia, conidiogenous cells and conidiophores were obtained from

TABLE I. GenBank accessions used in the molecular phylogenetic analyses of *Rhexocercosporidium* spp.

	ITS[7]	β-tubulin[7]	Percent similarity of β-tubulin sequence to RRD1[8]	Origin
Rhexocercosporidium RRD1 (DAOM 235605)[1]	DQ249992	DQ457119	—	Canada
Rhexocercosporidium RRD3[1]	DQ249993	DQ457120	100	Canada
Rhexocercosporidium KAML3 (DAOM 235603)[1]	DQ249995	DQ457118	100	Canada
Rhexocercosporidium F-ASH92 (DAOM 235604)[1]	DQ249994	DQ457121	100	Canada
Rhexocercosporidium carotae DAOM 226960[2]	*AF487894*[7]	DQ457117	94.1	Canada
Rhexocercosporidium carotae DAOM 229433	*AF487895*[7]	—	—	Norway
Rhexocercosporidium carotae PRI 784[3]	DQ526377	DQ526374	94.1	Netherlands
Rhexocercosporidium carotae PRI 787[3]	DQ526378	DQ526375	94.1	Sweden
Rhexocercosporidium sp. DSE48.1b[4]	DQ303121	—	—	Switzerland
Euascomycete OOO15[5]	*AJ246143*[7]	DQ457123	92.6	UK
Euascomycete OOO36[5]	*AJ246144*[7]	DQ457122	93.1	UK
Phialophora gregata, type A (98G1-3)[6]	DQ459386	DQ457124	89.6	USA

[1] Isolates of the rusted root causal agent obtained from diseased ginseng roots from the provinces of Ontario (RRD1, RRD3) and British Columbia (KAML3, F-ASH92). RRD1 (DAOM 235605), F-ASH92 (DAOM 235604) and KAML3 (DAOM 235603) have been deposited with the Canadian Collection of Fungal Cultures (CCFC), Ottawa, Ontario.

[2] Provided by C. Babcock.

[3] Provided by Dr J. Köhl

[4] Provided by Dr L. Wick.

[5] Provided by Dr A. Osbourn.

[6] Provided by Dr C. Grau.

[7] Accession numbers in italics were extracted from GenBank; all others were deposited to GenBank by the author during this study.

[8] Similarities in sequence to that of RRD1, as determined by the Martinez/Needleman-Wunsch alignment (Megalign, DNASTAR).

the same preparations with a Nikon digital camera (model DXM 1200) attached to a Zeiss Axioskop 2 Plus microscope equipped for direct interference contrast. Images were processed with Nikon ACT-1 software (version 2.12) and PaintShop Pro 5 (Jasc Software, Minneapolis, Minnesota).

Effect of pH on mycelial growth.—Molten V8 agar (containing 20% V8 juice clarified by centrifugation) was prepared with various sodium phosphate-citrate buffer mixtures to provide pH values of 3, 5, 6 and 7. Four replicate cultures of each pH treatment were prepared for each of ginseng *Rhexocercosporidium* isolate RRD1 and *R. carotae* DAOM 226960. After inoculation with 5 mm agar plugs, cultures were incubated at 18 C without light for 21 d. Radial measurements were made on agar plates after 7, 14 and 21 d. For each isolate the significance of effects of pH on radial growth was determined with ANOVA, with mean separation by Tukey's test (XL-Stat, Addinsoft, Paris, France).

DNA extraction, PCR amplification and sequencing.—DNA was extracted from washed mycelial mats, obtained from 2–4 wk old V8 broth cultures of fungi (TABLE I), with DNeasy Plant Mini kits (QIAGEN, Mississauga, Ontario, Canada). Excised mycelium was placed in sterile 1.5 mL microcentrifuge tubes then either ground with a sterile micropestle in the presence of liquid N_2, or homogenized in the presence of buffer AP1 (QIAGEN) and sterile zirconium oxide beads (1 mm diam, Reeleder et al 2003) using a Retsch MM301 mixer mill. The kit protocol was modified to provide 30 min rather than 10 min incubation of frozen or homogenized mycelium in buffer AP1. Extracts were eluted in buffer AE and stored at −20 C.

DNA in extracts was amplified with two oligonucleotide primer sets. One of these targeted the internal transcribed spacer regions (ITS 1 and ITS 2) of ribosomal DNA, including the intervening highly conserved 5.8S gene. The second primer set was used to amplify a portion of the β-tubulin gene.

ITS region amplification was carried out with the ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) / ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primer set (White et al 1990) with amplification conditions as follows. After an initial denaturing period of 60 s at 95 C, template DNA was amplified for 30 cycles (denaturing at 94 C for 60 s, annealing at 52 C for 30 s, and extension at 72 C for 60 s), followed by a final extension period of 7 min at 72 C. Reactions were cooled to 4 C before freezing at −20 C. Each 50 μL reaction consisted of 31.2 μL of sterile molecular-grade water, 5 μL of 10× PCR buffer, 5 μL of 25 mM $MgCl_2$, 20 μg bovine serum albumin (BSA) (1 μL), 1 μL of 10 mM dNTP solution (Invitrogen, Burlington, Ontario), 0.4 μL each of 50 mM solutions of ITS4 and ITS5 oligonucleotides (Invitrogen), 1 μL of 2.5 U μL^{-1} Jump-Start Taq DNA polymerase (Sigma-Aldrich, Oakville, Ontario), and 5 μL of extract. All reagents were obtained from Sigma-Aldrich unless otherwise indicated. In initial

tests a positive control was provided with 5 μL of extract from a culture of *C. destructans* f. sp. *panacis* (Seifert et al 2003) as the DNA template. In negative controls extract was replaced with 5 μL of sterile water. All PCR reactions were carried out with an Eppendorf Mastercycler (Brinkman, Mississauga, Ontario). PCR products were examined electrophoretically with 1.5 % molecular grade agarose or 3% NuSieve GTG agarose (Cambrex, East Rutherford, New Jersey) in 1× TAE buffer. Gels were run 20 min at ca. 8 V cm^{-1}, or 80 min at 5 V cm^{-1}. PCR products were sequenced with an Applied Biosystems 3730 Analyzer employing BigDye™ Terminator chemistry. To ensure sequence fidelity complementary sequencing was carried out separately with the ITS5 and ITS4 oligonucleotides.

The β-tubulin region was amplified with the primer set tub2F (5′-TGACCTGCTCTGCCATCTTG-3′) / tub2R (5′-ATACCCTCACCAGTGTACC-3′) (Hirsch et al 2000) with amplification conditions as follows. After an initial denaturing period of 3 min at 94 C, template DNA was amplified for 35 cycles (denaturing at 94 C for 45 s, annealing at 60 C for 45 s, and extension at 72 C for 60 s), followed by a final extension period of 7 min at 72 C. Reactions were cooled to 4 C before freezing at −20 C. Reagent concentrations and post-PCR operations were as described above. Complementary sequencing was carried out as described above, with the tub2F and tub2R primers. The annealing temperature of 60 C was selected after analysis of a preliminary set of reactions where a gradient of annealing temperatures (57−64 C) was evaluated for relative production of PCR product.

Molecular phylogenetic analysis.—Sequences obtained by the above procedures were deposited in GenBank (TABLE I) and were combined with selected pre-existing GenBank sequences in these analyses. Sequence data were aligned with Clustal W, as implemented in MegAlign (DNASTAR, Madison, Wisconsin). Alignments were submitted to TreeBASE (Piel et al 2003, IDS1758). Separate alignments first were carried out with each region (ITS and β-tubulin); each alignment then was imported into PAUP* (version 4.0b10, Swofford 2003) for analysis. Heuristic searches were run with the parsimony optimality criterion, with stepwise addition and with gaps treated as a fifth base. Bootstrap analyses (2000 replicates) subsequently were performed with the above settings. Partition-homogeneity tests, as implemented in PAUP*, were carried out to determine whether ITS and β-tubulin data could be combined before additional parsimony analyses. Resulting trees were exported to TreeView (Page 1996), rooted with the outgroup *Phialophora gregata* 98G1-3 (for ITS and β-tubulin datasets), and edited for clarity (Hall 2004).

RESULTS

Colony morphology.—Ginseng *Rhexocercosporidium* isolates appeared to be identical with respect to colony morphology. Therefore isolates RRD1 and KAML3 were used as representative cultures. On V8 agar, 2 wk old colonies (approx 43 mm diam) of ginseng *Rhexocercosporidium* isolate RRD1 and KAML3 were gray olivaceous (Rayner 1970), with an outer white margin (4–5 mm wide; FIG. 1); in V8 broth and on MRBA colonies were olivaceous gray. In V8 broth RRD1 and KAML3 colonies coalesced and covered the surface of the dish. The reverse of agar colonies was olivaceous black.

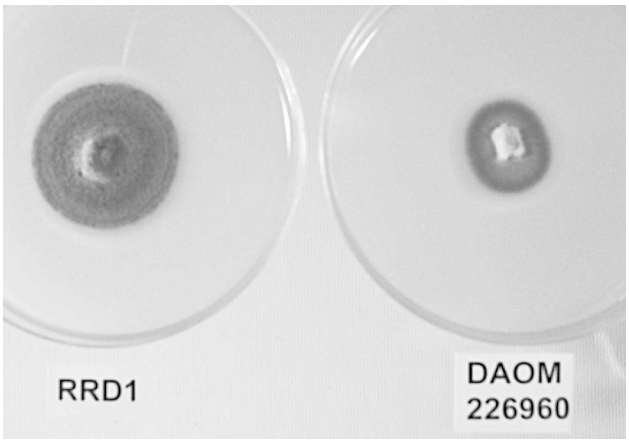

FIG. 1. Colonies of ginseng *Rhexocercosporidium* isolate RRD1 (*R. panacis*) and *R. carotae* DAOM 226960 on V8 agar, after 14 d growth at 18 C. Note differences in colony size.

Colonies of *R. carotae* DAOM 226960 in V8 broth were olivaceous buff when young, becoming iron gray to greenish black with age. In contrast to RRD1 and KAML3, *R. carotae* colonies in V8 broth tended not to coalesce or cover the entire dish surface. On V8 agar 2 wk old colonies (approx 24 mm diam) of *R. carotae* were in the main gray olivaceous, with a whitish to pale greenish gray center and a white margin (4–5 mm). The reverse was olivaceous gray to olivaceous black. *R. carotae* isolates PRI 784 and 787 were similar (TABLE I). *Rhexocercosporidium* DSE48.1b was white to lavender gray in V8 broth when young, pale purplish gray with age. Colonies on V8 were pale olivaceous gray to glaucous gray.

Conidiophores and conidiogenous cells.—Conidiogenous cells (5–20 × 2–4 μm) of *Rhexocercosporidium* RRD1 were most commonly integrated into vegetative hyphae and were clavate or cylindrical (FIG. 2). Conidiophores, when present, were intercalary and gave rise to a single conidiogenous cell. Conidia were blastic in origin; however, when mature, they were connected to the conidiogenous cell by a short, narrow cell. By contrast, conidiophores were common in *R. carotae* DAOM 226960. They often were intercalary and short (up to 59 μm long), usually consisting of one or more hyphal cells plus a clavate or cylindrical conidiogenous cell (5–20 × 2–4 μm) (FIG. 2). Intercalary conidiogenous cells (10–19 × 2–5 μm) also arose directly from vegetative hyphae.

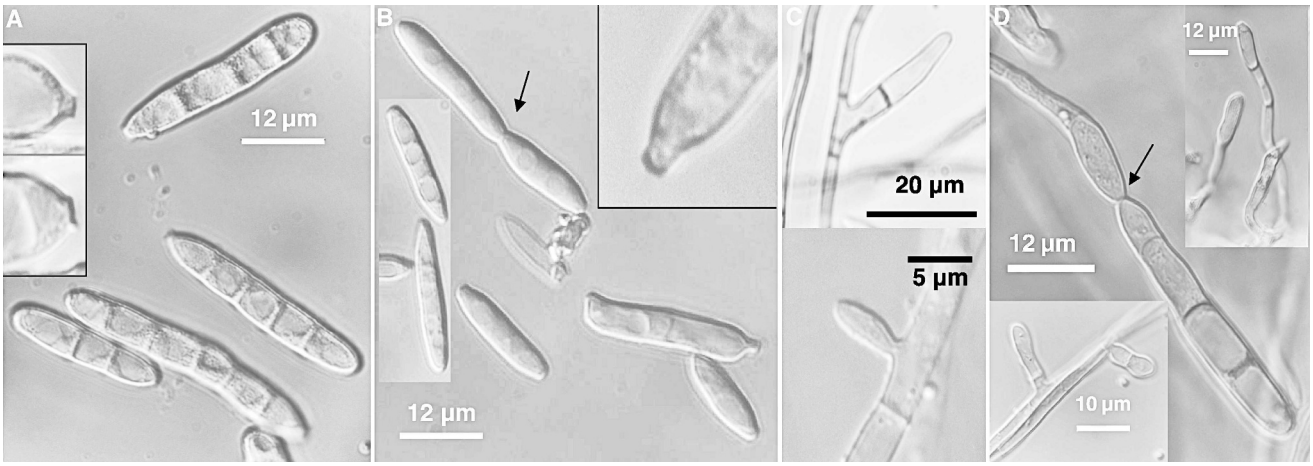

FIG. 2. Conidia, conidiophores and conidiogenous cells of *Rhexocercosporidium* spp. A. Conidia of *R. carotae*; insert (black border) shows close-up (not to scale) of collar and collar remnants following secession from conidiogenous cell. B. Conidia of ginseng isolates of *Rhexocercosporidium* (*R. panacis*). Black arrow indicates chain of two conidia. Insert close-up shows collar remaining after secession. C. Conidiophore and conidiogenous cell (top image) and conidiogenous cell (bottom) of *R. panacis*. D. Conidiophores and conidiogenous cells of *R. carotae*. Black arrow denotes conidium arising from conidiogenous cell. White and black bars represent scales.

Conidial size and shape.—Conidia of the ginseng *Rhexocercosporidium* RRD1 and *R. carotae* DAOM 226960 were compared. Conidia of the ginseng *Rhexocercosporidium* were 18.6 ± 0.4 (SE) × 2.8 ± 0.1 µm (n = 100), and 96% were 0–1-septate. Conidia were cylindrical or subcylindrical, straight and hyaline, and often rounded at the apex but with a basal truncate scar (FIG. 2). The remnant tissue of a subtending cell, in the form of a frill or collar was often attached to the scar (FIG. 2). Chains of two or more conidia often were present; if so, the conidia were separated by narrow neck. Conidia separated after the fracture of the neck, leaving collar-like remnants on both spores.

Conidia for *R. carotae* DAOM 226960 (n = 100) were 30.9 ± 0.6 × 5.1 ± 0.1 µm; 83% of conidia had more than 1 septum, and 57% had 3–5 septa. Chains of conidia were not observed. Due to the larger size of these conidia (compared to those of the ginseng *Rhexocercosporidium*), the collar-like remnants (Shoemaker et al 2002) were more readily observed (FIG. 2A). They were clearly fragments of tissue and not integral to the conidium structure. The Student's *t* test for independent samples (Satterthwaite's method, XL-Stat) was used to compare conidia of the two isolates; both length and width were found to be significantly ($P < 0.0001$) different for the two isolates.

Conidia of isolate DSE48.1b were 7.3 ± 0.3 × 2.0 ± 0.0 µm (n = 60), significantly ($P < 0.0001$) shorter and narrower than those of either RRD1 or DAOM 226960. Further, detached conidia of isolate DSE48.1b lacked the basal frill or collar observed on conidia of the other two isolates.

Effect of pH on radial growth.—Ginseng isolate RRD1 and *R. carotae* DAOM 226960 did not differentially react to pH. For both isolates increasing the agar pH to 7 resulted in a decrease in growth compared to growth at pH 5 or 6 (TABLE II).

Molecular phylogenetic analysis.—Ten single-spore isolates (six from British Columbia and four from Ontario) of the ginseng *Rhexocercosporidium* were obtained and the ITS regions were sequenced as described above. When aligned the sequences were found to be identical. Four isolates (two from British Columbia KAML3 [deposited with the Canadian Collection of Fungal Cultures, Ottawa, Ontario, as DAOM 235603] and F-ASH92 [DAOM 235604]) and two from Ontario (RRD1 [DAOM235605] and RRD3) were selected and used in phylogenetic analyses and in morphological comparisons with other *Rhexocercosporidium* isolates. Other fungi were selected for comparison to the ginseng *Rhexocercosporidium* on the basis of BLAST searches of GenBank. These included *R. carotae* DAOM 226960 (Shoemaker et al 2002), *Rhexocercosporidium* sp. DSE48.1b (Kohlmeier et al 2005), unidentified euascomycetes OOO15 and OOO36 (Carter et al 1999), and isolate 98G1-3 of *Cadophora gregata* (anamorph: *Phialophora gregata*; Harrington and McNew 2003, Hughes et al 2002). These isolates were obtained and the ITS region sequenced as described above. Similar sequence data for *R. carotae* DAOM 229433 was obtained from GenBank. ITS region sequence data of these cultures were aligned and compared with the MegAlign implementation of ClustalW. Ginseng *Rhexocercospor-*

TABLE II. Effect of pH on radial growth of *Rhexocercosporidium* spp.[1]

pH (nominal)	pH (actual)[2]	RRD1[3]		DAOM 226960[4]	
5	4.7	21.6	a	11.6	a
6	5.4	21.6	a	11.7	a
7	6.6	16.7	b	8.9	b
	Pr > F	0.0002		0.0005	

[1] Colony radii (mm) were determined after 14 d growth on V8 agar at 18 ± 1 C. Radii values followed by different letters are significantly different from others in the same column according to Tukey's (HSD) Test (alpha = 0.05).

[2] Actual pH of agar media was determined by inserting probe into slurry of macerated pieces of agar in water. pH values thus obtained were slightly lower than the nominal values predicted on the basis of the buffer mixtures used.

[3] Ginseng *Rhexocercosporidium* isolate RRD1 (DAOM 235605).

[4] *Rhexocercosporidium carotae* DAOM 226960.

idium isolates were 100% identical to one another and 97% similar to the *R. carotae* isolates; these two groups differed by 8 nucleotides in the ITS1 region and by 4 nucleotides in the ITS2 region. The *R. carotae* isolates were 99–100% similar to one another. Other fungi evaluated were less similar to the ginseng *Rhexocercosporidium* and *R. carotae* (Reeleder et al 2006).

The isolates also were amplified with a β-tubulin primer set and sequenced. Again the ginseng *Rhexocercosporidium* isolates were 100% identical to one another, 94% similar to *R. carotae* and less similar to other fungi (TABLE I). The ginseng *Rhexocercosporidium* isolates and the *R. carotae* isolates differed by 12 nucleotides over the β-tubulin sequence. Although the ITS primer set was used successfully to amplify the ITS1-5.8S-ITS2 region of isolate DSE48.1b, amplification of the β-tubulin region of this isolate was not successful. Further tests were done with two additional β-tubulin primer sets (O'Donnell et al 1998, Slippers et al 2004); however neither was successful in producing a detectable β-tubulin product from DNA of *Rhexocercosporidium* DSE48.1b. Consequently this isolate was excluded from β-tubulin analyses.

For ITS alignment data 12 taxa provided 515 characters to the data matrix. Of these 438 characters were constant, 42 characters were parsimony uninformative and 35 characters were parsimony informative. One of the four most parsimonious ITS trees retained is shown (FIG. 3). *R. carotae* and the ginseng *Rhexocercosporidium* (*R. panacis*) were placed in separate distinct clades in all four retained trees, well supported by bootstrap analysis (2000 replications). These two clades were well separated from *Rhexocercosporidium* DSE48.1b and the unidentified isolates OOO15 and OOO36. For the β-tubulin data 10 taxa contributed 202 characters to the data matrix. Of these 170 were constant, 13 characters were parsimony uninformative and 19 characters were parsimony informative. The most parsimonious trees resulting from analysis of β-tubulin data were similar to the ITS trees, particularly with respect to the placement of *R. carotae* and the ginseng *Rhexocercosporidium* (*R. panacis*) in distinct clades (trees not shown). A partition homogeneity test indicated that the ITS and β-tubulin data could be combined (P = 1.00). For the combined data 10 taxa contributed 716 total characters, Of these 617 were constant, 50 were parsimony uninformative and 49 were parsimony informative. One of the most parsimonious trees is shown (FIG. 4). Again *R. carotae* and the ginseng *Rhexocercosporidium* were sorted into distinct clades in all retained trees, well supported by bootstrap analysis.

TAXONOMY

The data indicate that the isolates from ginseng are distinct from the only species described previously in

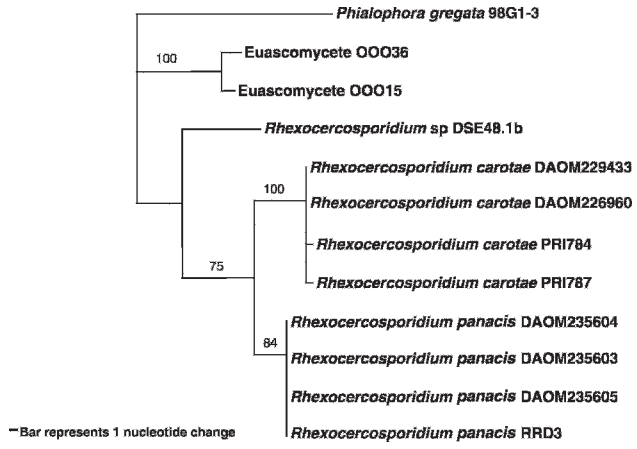

FIG. 3. Relationship of *Rhexocercosporidium carotae* and *Rhexocercosporidium panacis*, based on internal transcribed spacer region rDNA sequence data and maximum parsimony analysis. One of four retained trees; TL = 93, CI = 0.87, HI = 0.13, RI = 0.85, RC = 0.74. Bootstrap support values greater than 50% for 2000 replications are shown at the nodes. The tree was rooted to *Phialophora gregata* 98G1-3.

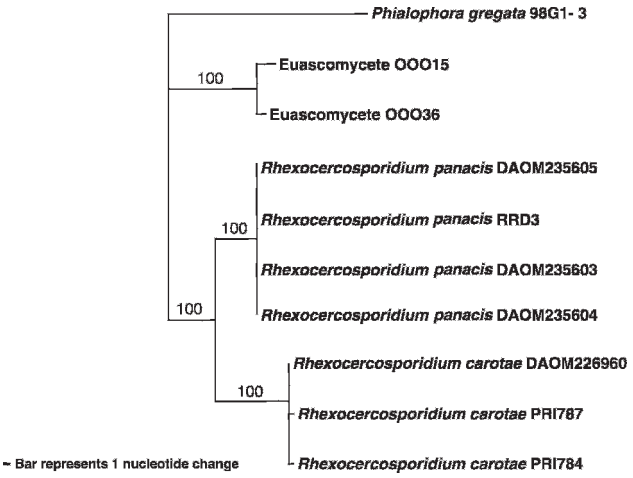

FIG. 4. Relationship of *Rhexocercosporidium carotae* and *Rhexocercosporidium panacis*, based on maximum parsimony analysis of combined internal transcribed spacer region rDNA and β-tubulin sequence data. One of two retained trees; TL = 105, CI = 0.98, HI = 0.02, RI = 0.98, RC = 0.96. Bootstrap support values greater than 50% for 2000 replications are shown at the nodes. The tree was rooted to *Phialophora gregata* 98G1-3.

the genus *Rhexocercosporidium*. The sexual state was not observed in culture or on the ginseng host, and it has not been observed for *R. carotae*. Therefore the ginseng isolates are being described as a new anamorphic species.

Rhexocercosporidium panacis Reeleder sp. nov.
FIGS. 1, 2

Coloniae in agaro V8 appressae, initio albae, tum fumoso-olivaceae, infra atro-olivaceae, margine angusto albo. Lente crescentes; 43mm diam post 14 dies ad 18 gradibus caloris in agaro V8 (incrementum diametri circa 1.6 mm per diem ad 16 gradibus caloris). Hyphae 1.8–3.8 μm diam. Conidia ex culturis in iure V8 cultis (12.5–)17.8–19.4(–30) × (2.5–)2.7–3(–5) μm diam, plerumque 0–1-septata, interdum 2-septata. Conidia cylindrica aut subcylindrica, recta, hyalina, saepe ad apicem rotundata, sed ad basin cicatrice truncata, ad quam fimbriae minutae adiunctae sunt. Conidiophora plerumque absentia; praesentia sine ramis aut cum ramis sympodialibus, vix dissimilia hyphis vegetativis. Cellulae conidiogenae (12–59 × 2–4 μm) plerumque terminales, clavatae aut cylindricae, cicatricibus conidialibus. Secessio conidiorum a cellulis conidiogenis rhexolytica.

Colonies on V8 agar are appressed, white at first, then turning olivaceous gray (Rayner 1970), olivaceous black from below, with a narrow white margin. Slow-growing; 43 mm diam after 14 d at 18 C on V8 agar (radial growth approx. 1.6 mm/d at 16 C). Hyphae 1.8–3.8 μm wide. Conidia from V8 broth cultures (12.5–)17.8–19.4(–30) × (2.5–)2.7–3(–5) μm, mainly 0–1-septate, occasionally 2-septate. Conidia cylindrical or subcylindrical, straight, hyaline, often rounded at the apex but with a basal truncate scar, to which is often attached remnant tissue of a subtending cell, in the form of a frill or collar. Conidia often catenulate, forming acropetal chains. Conidiophores mainly absent; when present unbranched or with sympodial branching, little differentiated from vegetative hyphae. Conidiogenous cells (12–59 × 2–4 μm) mostly terminal, clavate or cylindrical, possessing conidial scars. Secession of conidia from conidiogenous cells is rhexolytic.

HOLOTYPE: DAOM 235605 (*Rhexocercosporidium* sp isolate RRD1), isolated from roots of cultivated *Panax quinquefolius* collected from research plots at the Delhi research farm (42°52′N, 80°33′W) of Agriculture and Agri-Food Canada, Norfolk County, Ontario, Canada. 2 Jun 2005.

Specimens examined.—CANADA, ONTARIO: Norfolk County, roots of cultivated *Panax quinquefolius*, 2005, *R. Reeleder*, DAOM 235605 (*Rhexocercosporidium* sp. isolate RRD1). CANADA, ONTARIO: Norfolk County, roots of cultivated *Panax quinquefolius*, 2005, *R. Reeleder*, *Rhexocercosporidium* sp. isolate RRD3. CANADA, BRITISH COLUMBIA: roots of cultivated *Panax quinquefolius*, 2005, *R. Reeleder*, DAOM 235603 (*Rhexocercosporidium* sp. isolate KAML3). CANADA, BRITISH COLUMBIA: roots of cultivated *Panax quinquefolius*, 2005, *R. Reeleder*, DAOM 235604 (*Rhexocercospor-Rhexocercosporidium* sp. isolate F-ASH92).

Etymology.—*Panacis* is chosen to reflect the host from which the fungus has been isolated.

DISCUSSION

R. carotae, the causal agent of black rot of carrot (*Daucus carota* L.), first was described as *Acrothecium carotae* (Årsvoll 1965). Effects of temperature on fungal growth and conidium size, as well as various studies on pathogenicity and host range, subsequently were determined (Årsvoll 1971). The fungus later was transferred to *Pseudocercosporidium* (de Hoog and Van Oorschot 1985) and then to *Rhexocercosporidium* by Braun (1994), who erected this new anamorphic genus to accommodate the carrot pathogen. The genus is characterized by conidiophores that are little differentiated from vegetative hyphae, conidia obovoid to cylindrical, with rhexolytic spore secession (Braun 1994, Shoemaker et al 2002). Conidia were reported to be 25–45 × 5–6.5 μm (Shoemaker et al 2002). This is consistent with data reported here for this species. The sexual state has not been reported, but Shoemaker et al (2002) used an analysis of ITS and 18S sequences to place *R. carotae* within the Leotiomycetes, a class within the Pezizomycotina that contains a number of plant pathogens, including species of *Erisyphe, Botryotinia* and *Sclerotinia*. Com-

parisons of *R. carotae* ITS sequence data with GenBank data found that it was closely related to the oat root euascomycetes OOO15 and OOO36 (Carter et al 1999; Shoemaker et al 2002). BLAST (Altschul et al 1990) searches of GenBank accessions with sequences of the ginseng *Rhexocercosporidium* found similar relatedness (Reeleder and Hoke 2005, Reeleder et al 2006). These similarities were confirmed when isolates of a number of these fungi were obtained from collaborators and resequenced. β-tubulin sequence data confirmed these relationships.

In addition to the characteristic black rot of stored carrots, *R. carotae* also damages carrot foliage and causes a damping-off of carrot seedlings. It is uncertain whether *R. panacis* has similar capabilities. Although the black rot of stored carrots is generally shallow and does not penetrate deeply into the root, Årsvoll (1965) believed that wounded roots might be more extensively damaged. A number of umbelliferous species may be susceptible to *R. carotae* (Årsvoll 1971). It has been shown that, in inoculation tests, *R. panacis* can reproduce the symptoms of rusted root (Reeleder et al 2006), although the host range of this new species is not yet determined. Preliminary experiments indicate that *R. carotae* is not pathogenic on non-wounded ginseng roots and that *R. panacis* will not attack carrot.

Rhexocercosporidium isolate DSE48.1b is distinct from both *R. panacis* and *R. carotae*. Detached conidia of isolate DSE48.1b did not appear to have the basal frill characteristic of other members of the genus; this was clearly present on conidia of *R. panax* and *R. carotae*. ITS sequence data for DSE48.1b are distinct from those of the *Rhexocercosporidium* isolates from ginseng and carrot. Although β-tubulin sequence data could not be obtained for DSE48.1b, it appears that this isolate has little in common with other currently described members of the genus. The status of the euascomycete isolates OOO15 and OOO36 is unclear; however they appear to be more distantly related to *R. carotae* and *R. panacis* than is *Rhexocercosporidium* DSE48.1b.

The genetic sequence and morphological data presented here clearly show that *R. panacis* and *R. carotae* are distinct, although they share the rhexolytic secession of conidia and the resulting frills on either side of the conidial scar that result from the rhexolytic secession characteristic of the genus. It was reported previously that, although both the ginseng *Rhexocercosporidium* and *R. carotae* exhibit maximum growth rates at approx 18 C, the ginseng *Rhexocercosporidium* grows considerably faster, with a growth rate of 1.61 ± 0.03 (SE) mm/d compared to 0.69 ± 0.03 mm/d for *R. carotae* (Reeleder et al 2006). However pH does not appear to differentially affect *R. panacis* and *R. carotae*. Future research might affect the taxonomic disposition of *Rhexocercosporidium* and the allied isolates discussed here (Shoemaker et al 2002), nonetheless the current data suggest that the ginseng isolates are sufficiently distinct from *R. carotae* to support the establishment of a new anamorphic species, *R. panacis*.

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