Postharvest rot by *Coprinus psychromorbidus* on apples and pears in cold storage in British Columbia

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A sterile, low-temperature tolerant basidiomycete consistently isolated from apples in cold storage, is identified as *Coprinus psychromorbidus*, Redhead and Traquair. The fungus grew well at 10°C and produced white, cottony to woolly colonies on malt agar and potato dextrose agar. Hydrogen cyanide production was weak. Compatibility of dikaryotic mycelium of this fungus with monokaryotic isolates of *C. psychromorbidus* causing snow mold of alfalfa and winter wheat, is genetical evidence for their conspecificity.

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Un champignon basidiomycbte stbrile, tolérant les basses températures et isolé régulièrement Á partir de pomme entreposb au froid, a été identifié comme *Coprinus psychromorbidus*, Redhead et Traquair. Le champignon montre une bonne croissance à 10°C et produit des colonies blanche d'aspect cotoneux Á laineux sur de l'agar de malt et de l'agar de pomme de terre et de dextrose. La production de cyanure d'hydrogbne est faible. La compatibilitb du mycellium dikaryote de ce champignon avec des isolats monokaryote de C. *psychromorbidusqui* cause la moisissure nivale de la luzerne et du blé d'hiver, constitue une bvidence génétique de leur conspécificité.

Introduction

Coprinus rot of apples in cold storage has become a serious problem in the Okanagan Valley of British Columbia. Golden Delicious, McIntosh, Spartan, Newton and Red Delicious apples are all affected (Meheriuk and McPhee 1984). The dry, dark brown lesions with tan centers are variable in appearance but in all cases, advanced stages of rot are marked by masses of cottony, white mycelium that cover the surface of infected apples and packing materials. The same fungus causes a storage rot of d'Anjou pears in British Columbia (Meheriuk and McPhee 1984). In Oregon, the pear rotting fungus was identified simply as a Coprinus sp. in the *urticicola* complex and was shown to be conspecific with the low-temperature basidiomycete (LTB) that causes cottony snow mold of grasses and winter crown of alfalfa (Spotts et *al.* 1981). Taxonomic revision of Coprinus species in the section *Herbicolae* showed that the snow mold LTB was a new species, *Coprinus psychromorbidus* Redhead & Traquair (Redhead and Traquair 1981). The precise identity of the apple-rotting LTB has not been published.

The apple-rotting basidiomycete that grows at 10°C is described here and identified as C. *psychromorbidus* based on the results of dikaryon-monokaryon mating tests.

Materials and methods

The fungus (78-2) used in this study was isolated by W. McPhee from rotted Golden Delicious apples in cold storage in Summerland, B.C. and was stored in the culture collection at the Lethbridge Research Station as LRS-070.

Cultures for the description of growth and colony features were grown in the dark on malt extract agar (MA) as outlined by Nobles (1965) and on Difco potato dextrose agar (PDA) in 9-cm plastic petri plates at 10 and 22°C.

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Radial growth was measured weekly for six weeks. The fungus was also cultured on PDA and MA on the laboratory bench at 22+ 1"C under alternating dark and fluorescent light conditions (8 h/da at 9 $uEM^{-2}s^{-1}$).

Mycelium was examined weekly using squash-mounted samples in distilled water or in 5% KOH and 1% phloxine B. Drawings were made with the aid of a camera lucida.

Tests for the production of extracellular polyphenoloxidase were performed by growing the fungus on gallic acid agar and by dropping a freshly prepared solution of alcoholic gum guaiacum on the margin of 21-day-old colonies (Nobles 1965). Production of brown diffusion zones and blue pigment in the gallic acid and gum guaiacum tests. respectively indicated a positive reaction. The ability to produce hydrogen cyanide (HCN) in culture was determined by observing color changes in alkaline sodium picrate on PDA in Conway diffusion dishes (Chalkley and Millar 1982; Lebeau and Hawn 1963). A change from yellow to orange or red indicated a positive reaction.

The margins of 10-day-old colonies of four monokaryotic tester isolates of C. *psychromorbidus* (DAOM 175227-1, 175227-2, 175227-7, and 175227-13) grown separately on PDA in 9-cm plastic petri plates were inoculated with dikaryotic mycelium from a 10-day-old culture of the unidentified baaidiomycete grown on PDA at 10°C in the dark. In addition, the tester rnonokaryons were paired with dikaryotic colonies of C. psychromorbidus (DAOM 175227 and DAOM 175229) as a check for mating potential. Hyphae from the colony margin of the tester distal to the unidentified dikaryon were examined 7 and 14 days after pairing, by mounting in KOH and phloxine as previously described and by looking for the presence of clamp connections and binucleate cells with the aid of phase contrast light microscopy.

Results and discussion

Cultural characteristics

Colony growth was moderate (4.5 mm/da) to slow (1.6 mm/da) at 10°C and 22°C respectively. The margin of the ad-

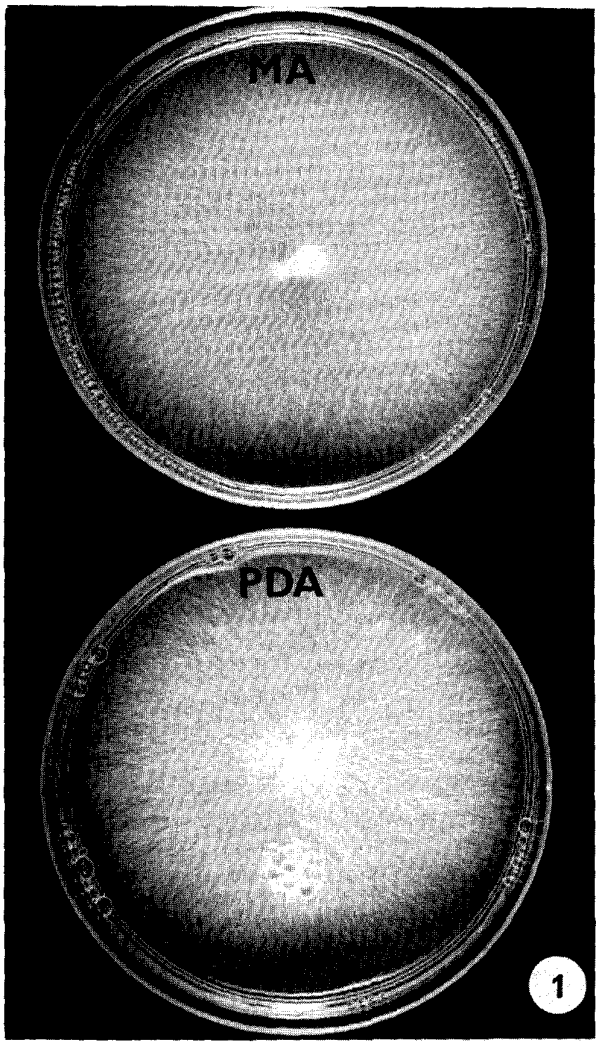

Figure 1. Woolly aerial mycelium of 3-week-old colonies of the low temperature basidiomycete **(78-2)**, ***Coprinus psychromorbidus*** on malt agar (MA) and potato dextrose agar (PDA) × **0.8.**

vancing colony was even, thin and appressed or cottony to woolly after 3 weeks. Aerial mycelium was white and cottony to woolly at 3 weeks (Fig. 1); after 6 weeks the aerial of colonies was unchanged in color on PDA but on **MA** in the light, a yellow pigment diffused into the agar. After 3-5 days exposure to fluorescent light on the lab bench, this pigment turned pink to purple. The odor of cultures was mushroom-like or not distinctive. No fruiting or sclerotium production was observed in culture on **PDA** or **MA** media during the 6-week study period.

Tests for extracellular polyphenol oxidases using gum guaiacum solution were positive (blue pigment appearing within 5 min.) as were tests using gallic acid medium (brown diffusion zones were produced in 10-14 days). Results were weakly positive after 30 days in tests for HCN using alkaline sodium picrate (color change from yellow to orange).

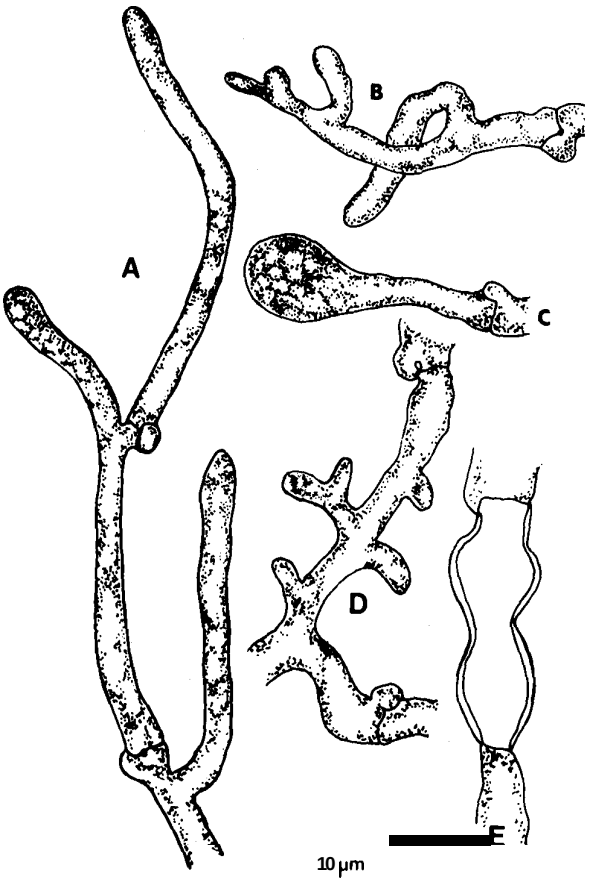

Figure **2.** Thin-walled generative hypha with clamp connections (A), antleroid branch (B), allocyst-like branch (C), gnarled submerged hypha (D) and thick-walled swollen hypha **(E)** in 4-week-old PDA colonies of ***Coprinus psychromorbidus.*** isolate **78-2.**

Generative hyphae were hyaline and thin-walled with clamp connections at cross walls (Fig. **2A).** Hyphae in the advancing zone were 2.5-3.0 µm wide and sparsely branched, often at the site of a clamp connection. Hyphae on the agar surface and in the aerial mycelium were 2.5-4.0 µm wide and frequently branched. Scattered antleroid (Fig. 2B) and allocyst-like (Nobles 1965) branches (Fig. **2C)** were observed on the surface hyphae. The submerged hyphae (3.0-5.0 µm wide) were gnarled and contorted (Fig. 2D) with thin- and thick-walled swellings (Fig. 2E) that ranged in diameter from 5-6.5 µm.

Dikaryon-monokaryon mating??

The apple isolate, LRS 070 (78-2), from British Columbia dikaryotized all the monokaryotic tester isolates of C. ***psychromorbidus*** (Table 1). The colonies merged and a barrage-type (Traquair and Kennedy 1974) interaction with less dense mycelium (Fig. 3) was noted in the confrontation zone of pairings with one of the two mating types (Traquair 1980).

Dikaryotization of ***C. psychromorbidus*** mycelium in di-mon mating tests with the apple-rotting isolate, is genetical evi-

Table 1. Results of matings between monokaryotic tester isolates of Coprinus *psychromorbidus* and the dikaryotic isolate of a low-temperature basidiomycete from apples in cold storage.

C. *psychromorbidus* dikaryon	*C.* psychrornorbidus (DAOM 175227-) monokaryon			
	175227-1	175227-2	175227-7	175227-13
LRS 070 (78-2)	+*	+	+	+
DAOM 175227	+	+	+	+
DAOM 175229	+	+	+	+

* + denotes complete dikaryotization as recognized by presence of clamp connections and binucleate cells throughout the tester colony.

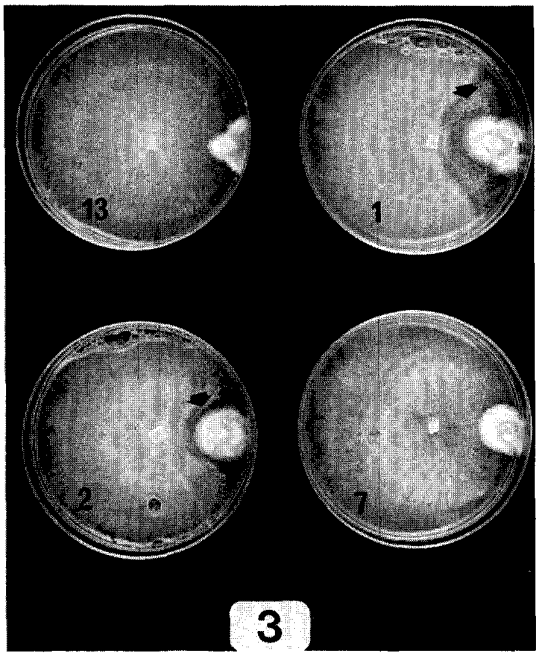

Figure 3. Dikaryon-monokaryon matings of the dikaryotic apple isolate (78-2) with monokaryotic tester isolates (-1,-2, -7,-13) of *Coprinus* psychromorbidus(DAOM 1752271. Note the barrage-like interaction with one mating type (arrows) x 0.4.

dence of their conspecificity. These results are also supported by similar growth characteristics of the fungi in culture. The white, cottony-woolly colonies that grow well at 10°C are typical of the *Coprinus* sp. rotting d'Anjou pears (Spotts et *al.* 1981) and of the non-sclerotial strains of *C. psychromorbidus* causing cottony snow mold of alfalfa, grasses and winter cereals (Traquair 1980).

Coprinus rot of apples and pears could be confused with tan-spot rot or fisheye rot caused by *Butlerelfia eustacei* Weresub & Illman (= *Corticium centrifugum* (Lev.) Bres.) except for the fact that the fungus causing coprinus rot grows better at 10°C than at 25°C (Meheriuk and W.J. McPhee 1984; Weresub and Illman 1980). Within 6-10 days on PDA, the *B. eustacei* produces characteristic cremaceous waxy granules or membranous patches which are the basidiome (Weresub and Illman 1980).

The psychrophilic apple isolate is a weak HCN producer but, previous work with C. *psychromorbidus* has shown that ability to produce HCN in culture varies with the isolate and cultural conditions (Traquair 1980; Ward et *al.* 1961). Although analysis of infected apple tissue for HCN was not done, previous work has shown that isolates of C. *psychromorbidus* (identified then as LTB) producing little to no HCN in culture, are the most aggressive on host plants (Gaudet 1986; Traquair 1980; Traquair and Hawn 1982; Ward *et al.* 1961).

Although sclerotial production was not observed for the apple isolate causing coprinus rot, black scllerotia were observed on the wood of storage crates infested with this fungus on pears in Oregon (Spotts et *al.* 1981). Sclerotia are likely to be significant survival propagules and sources of infection. Sterol inhibitors and dithiocarbamates were shown to reduce mycelial growth of the *Coprinus* sp. and, ziram applied to trees before harvest was shown to control coprinus rot in stored fruit (Spotts et *al.* 1981).

Washes with 5% borax have been recommended in the early literature as a means of disinfecting timber in cold stores that are in contact with food stuffs (Cartwright and Findlay 1958). It is interesting to note that symptoms of LTB snow mold on inoculated alfalfa have been reduced by applying borax solutions (Lebeau and Atkinson 1967). Good sanitation in the cold storage facility is an important part of control for coprinus rot of pome fruits.

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