d'Anjou Pear Decay Caused by a Low Temperature Basidiomycete

R. A. SPOTTS, Assistant Professor, Department of Botany and Plant Pathology, Oregon State University, Mid-Columbia Experiment Station, Hood River, OR 97031; J. A. TRAQUAIR, Research Scientist, Research Station, Agriculture Canada, Lethbridge, Alberta, Canada T1J 4B1; and B. B. PETERS, Experimental Biology Technician, Mid-Columbia Experiment Station, Hood River

ABSTRACT

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In 1979, a basidiomycete caused significant loss of d'Anjou pears in controlled atmosphere storage at Hood River, OR. By monokaryon-dikaryon pairings, the basidiomycete was shown to be conspecific with a *Coprinus* species in the *urticicola* complex that causes winter crown rot of alfalfa. Maximum radial mycelial growth was at 10 C. Tests were positive for extracellular polyphenoloxidase and hydrogen cyanide. Of 23 fungicides tested in vitro, sterol inhibitors and dithiocarbamates at 10 μg/ml significantly reduced mycelial growth. Ziram, applied to trees 10 days before harvest, provided significant control in stored fruit. This is the first report of pear fruit decay caused by a basidiomycete and the first record of this low temperature basidiomycete in the United States.

In 1979, a low temperature basidiomycete caused an estimated $115,000 loss of d'Anjou pears in commercial, controlled atmosphere storage in Hood River, OR. The isolated pathogen did not sporulate in culture, but growth characteristics were identical to those of the snow mold basidiomycete causing winter crown rot of alfalfa, several grasses, and overwintering cereals in Western Canada (3). Recently a sexual stage was reported for the snow mold basidiomycete (6). It forms basidiocarps morphologically similar to those of a fungus previously identified as *Coprinus urticicola* (Berk. & Br.) Buller associated with wheat and nettles (2). This report describes the symptoms of the basidiomycete rot of d'Anjou pear fruit, identifies the causal agent, and includes studies on chemical control.

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MATERIALS AND METHODS

Pathological studies. Tissue from lesion margins of decayed d'Anjou pear fruits was surface-sterilized in 0.5% NaOCl solution for 5 min, rinsed in sterile distilled water, plated on potato-dextrose agar (PDA) and incubated in darkness at 10 C.

d'Anjou pear fruits were inoculated by placing mycelium from 3-wk-old PDA cultures into 5-mm deep wounds. Six basidiomycete isolates recovered from infected pear fruits were used. Inoculated fruits were placed in a polyethylene-lined box, stored at −1 C, and examined monthly, at which time reisolations were made.

Compatibility studies. To determine the identity of nonfruiting isolates, dikaryotic-monokaryotic pairings (1) were performed on PDA (9-cm diameter petri plates). Three unknown isolates (dikaryotic as evidenced by presence of clamp connections) from pear fruit were paired with four 10-day-old monokaryotic isolates of the alfalfa *Coprinus*. The later isolates were obtained by isolating single spores from mature basidiocarps. After 7–10 day incubation at 22 C in darkness, samples of mycelium and agar (about 1 mm wide) were cut from the margin of the monokaryotic colony distal to the dikaryotic isolate. The mycelium was examined using the procedures of Nobles (5), ie, squash-mounted in equal parts of 5% KOH and 1% phloxine B and then examined for clamp connections and binucleate cells by using phase contrast microscopy.

Table 1. In vitro growth reduction of *Coprinus* induced by fungicides

Fungicide		Avg. percentage growth reduction [a] at fungicide concentration (μg/ml)		
Common name	Trade name and formulation	1	10	100
...	CGA 64251 10WP	100 a	100 a	100 a
fenapronil	Sisthane 25WP	51 c	100 a	100 a
bitertanol	Baycor 50WP	55 c	88 b	100 a
sodium-o-phenyl phenate	Steri-seal 22.6%	...	97 ab	100 a
triadimefon	Bayleton 50WP	35 d	95 ab	100 a
ziram	Ziram	74 b	92 ab	89 b
dichloran	Botran 75WP	28 def	93 ab	95 a
mancozeb	Dithane M45 80WP	35 d	71 cd	100 a
chlorothalonil	Bravo 500F	61 c	78 c	84 c
ferbam	Fermate 76WP	25 def	55 fg	100 a
...	Dikar 77WP	23 defg	61 ef	96 a
lime sulfur	Orthorix 27.5%	21 efgh	51 gh	100 a
iprodione	Chipco 26019 50WP	20 efgh	43 h	99 a
dinocap	Karathane 22.5WP	33 de	65 de	84 c
dodine	Cyprex 65WP	28 def	61 ef	72 e
captan	Captan 50WP	28 def	63 fg	84 c
vinclozolin	Ronilan 50WP	17 fghi	70 cde	78 d
benomyl	Benlate 50WP	12 ghij	29 i	80 cd
...	Topcop 11F	18 fghi	27 i	47 f
thiabendazole	Mertect 340F	7 ij	1 k	78 d
sulfur	THAT flowable sulfur	12 ghij	24 i	42 g
copper	Kocide 101 77WP	9 hij	11 j	15 h
metalaxyl	Ridomil 5WP	2 j	6 jk	18 h

[a] Numbers followed by the same letter within columns are not significantly different at $P = 0.05$ according to Duncan's new multiple range test.

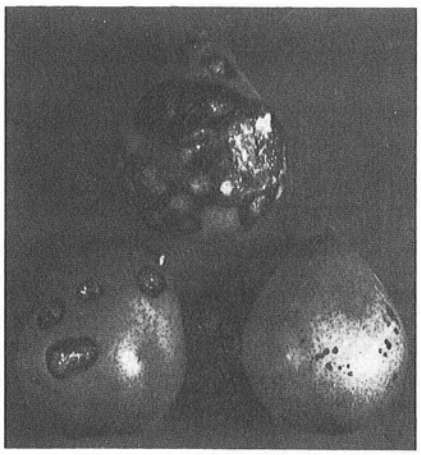

Fig. 1. Low temperature basidiomycete decay of d'Anjou pear fruit.

Temperature-growth relationships. Disks (4.0-mm diameter) from the margins of pear basidiomycete cultures were placed at the center of PDA plates (14.4 ml per 9-cm diameter plate) and grown in darkness at 0, 5, 10, 15, 20, and 25 C. Radial growth was measured weekly.

Colony morphology. Cultures were grown in the dark on PDA in 9-cm petri plates incubated at 10 and 15 C. Cultures were examined daily using squash-mounted samples of mycelium in 5% KOH and 1% phloxine B and observed by light microscopy with bright field and phase contrast optics.

Chemical tests. In order to further characterize the pear basidiomycete and compare it with the alfalfa *Coprinus*, tests for the production of extracellular polyphenoloxidases were performed by dropping a freshly prepared solution of alcoholic gum guaiacum (5) on the margin of 21-day-old colonies on PDA. Production of a blue pigment indicates a positive reaction. The ability to produce hydrogen cyanide in culture was determined by color changes in picric acid solution exposed to cultures on PDA in Conway diffusion dishes (4). A change from yellow to orange or red indicates a positive reaction.

Fungicide evaluation. Twenty-three fungicides (Table 1) and the antibiotics chloramphenicol, neomycin sulfate, vancomycin, and streptomycin were screened in vitro for activity against the pear basidiomycete as described previously (7). Two 4-mm-diameter plugs from 2-wk-old cultures were placed on each plate of PDA and fungicide-amended PDA (eight replicates per treatment). Colony diameters were measured after 14-day incubation at 10 C.

In field studies, d'Anjou trees in a commercial orchard with a history of basidiomycete rot were sprayed with registered fungicides that had significantly reduced mycelial growth in laboratory tests. These included mancozeb, 1.45 g a.i./L, and ziram, 1.8 g a.i./L. Treatments were applied to runoff (21 kg/cm^2) with a handgun sprayer to single-tree plots, six replications per treatment. About 16 L of spray were applied to each tree 10 days before harvest. All fruits, including those of untreated controls, were harvested on 10 September 1979, placed in polyethylene-lined boxes, stored at −1 C, and examined monthly for decay.

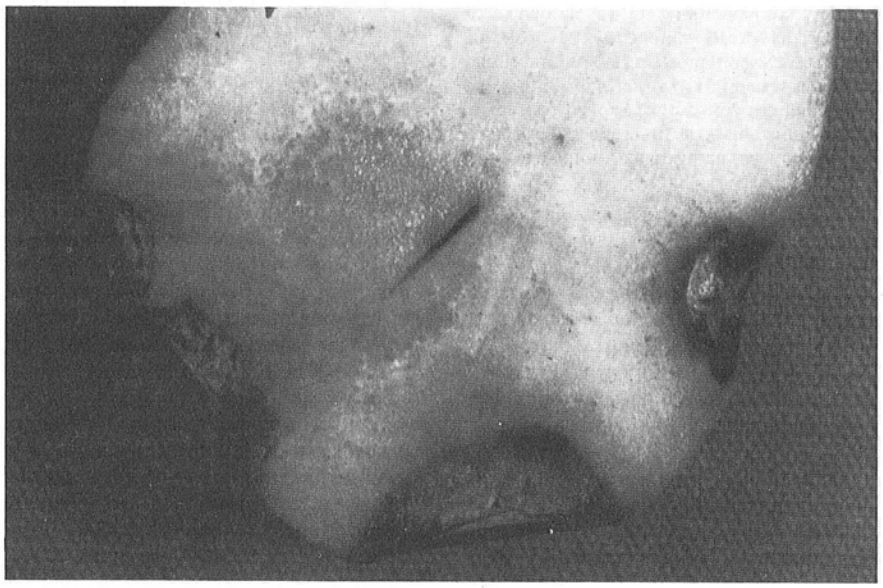

Fig. 2. Characteristic decay of d'Anjou pear flesh by low temperature basidiomycete. Penetration is not extensive, and decayed flesh is firm and dry.

Fig. 3. Mycelial spread of pear basidiomycete and secondary infection of d'Anjou pear fruit after 8-mo storage at −1 C.

RESULTS

Basidiomycete rot of d'Anjou pear fruit. In naturally occurring decay of d'Anjou pear fruits stored under commercial, controlled atmosphere conditions (2.5% oxygen, 1% carbon dioxide) at −1.1 C for 9 mo, lesions were circular and sunken with dark brown borders and lighter centers (Fig. 1). Lesion diameter ranged from 0.5 to 25 mm, and the number of lesions per fruit varied from 1 to 25. Infected tissue was firm and dry and seldom exceeded 5 mm in depth (Fig. 2). Occasionally, the entire fruit surface was decayed and rubbery, with decay extending into the flesh approximately 15 mm. Extensive white, raised mycelium frequently covered fruit surfaces, fruit wraps, and trays (Fig. 3).

Pathological studies. The basidiomycete was isolated consistently from naturally infected fruits. Typical lesions and fruit decay were produced in all 180 d'Anjou fruits inoculated with six isolates. Reisolation of the basidiomycete varied among isolates from 0 to 88% and averaged 46%, thus completing Koch's postulates.

Identification and compatibility

studies. When dikaryotic isolates of the unknown pear fungus were paired with monokaryotic isolates of *Coprinus* from alfalfa, the results indicated that the two basidiomycetes are conspecific. There was no evidence of antagonism, hyphal fusion occurred, and the *Coprinus* colony was dikaryotized as evidenced by the presence of clamps and dikaryotic cells.

Temperature-growth relationships. Optimum radial growth temperature of the pear basidiomycete was at 10 C, and no growth occurred at 25 C. Average radial growth after 3 wk at 0, 5, 10, 15, 20, and 25 C was 3, 14, 20, 16, 6, and 0 mm, respectively.

Colony and anatomic morphology. The advancing zone of the pear *Coprinus* colony grown on PDA at 10 C was even to uneven, thin and appressed to cottony. The aerial mycelium was white and formed at first a woolly to cottony mat that later (6 wk) became appressed and feltlike (Fig. 4). In older colonies, small (1–2 mm wide), white hyphal knots occasionally appeared on the surface and within the agar. The color of the culture medium did not change. The culture odor was fungoid. There was no evidence of sporophore development. Black sclerotial patches were observed on inoculated pear wood after 12 wk.

Hyphae were composed of binucleate cells that were hyaline and thin walled with clamp connections at the cross walls. Hyphae of the advancing zone were 1.6–4.0 μm wide and sparsely branched. Hyphae on the agar surface were 2.0–4.0(–5.5) μm wide and frequently branched. Occasionally, hyphae with coralloid or antlerlike branching are scattered in the surface mycelium. Submerged hyphae were 1.6–5.6(–8.0) μm wide, contorted and frequently branched. Slight thickening was evident, in addition to ovoid or subglobose intercalary swellings. Hyphae of the hyphal knots were thin walled, 1.6–8.0 μm wide and compactly interwoven. Sclerotial masses on wood consisted of compactly interwoven hyphae. The outer hyphae were 2.0–5.5 μm wide, thick walled, blackish, and refractile. The inner hyphae were 4.8–8.0 μm wide, hyaline, and thin walled.

Chemical tests. Tests for extracellular polyphenoloxidases were positive with gum guaiacum solution. Tests for the production of hydrogen cyanide were weakly positive after 30 days.

Fungicide evaluation. Of the fungicides tested in vitro, the experimental sterol inhibitors CGA 64251, fenapronil, bitertanol, and triadimefon were most effective (Table 1). Ziram and mancozeb, two registered fungicides that are commonly used in preharvest applications, were also effective in vitro. The benzimidazoles, benomyl and thiabendazole, as well as sulfur and copper reduced mycelial growth less than other fungicides. The antibiotics chloramphenicol (10 μg/ml), neomycin sulfate (100 μg/ml), vancomycin (200 μg/ml), and streptomycin (100 μg/ml) were generally ineffective.

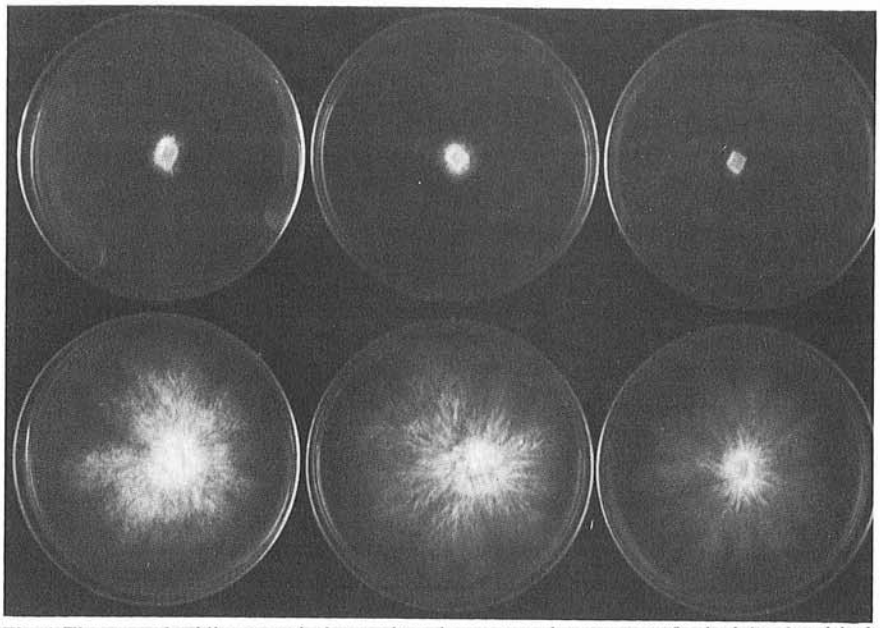

Fig. 4. Three pear basidiomycete isolates cultured on potato-dextrose agar for 1 wk (top) and 4 wk (bottom).

In orchard studies, ziram significantly ($P = 0.05$) reduced basidiomycete infections as later observed in storage, but control with mancozeb was not significant. The average decay of 600 fruits per treatment, stored 8 mo at −1 C, for ziram, mancozeb, and the unsprayed control was 7, 47, and 59%, respectively. In addition to orchard infection, secondary spread of the basidiomycete in cold storage resulted in decay of 6, 27, and 31% of ziram, mancozeb, and control fruits, respectively.

DISCUSSION

The basidiomycete that decays d'Anjou pears in storage is identical in growth and other diagnostic characteristics to the *Coprinus* that causes winter crown rot of alfalfa and snow mold to other crops (6). This is the first record of this low temperature basidiomycete in the United States and is also the first record of its occurrence on fleshy fruit of pear. Although the snow mold pathogen remained unidentified for a number of years, a sexual stage belonging to a species in the *C. urticicola* complex was recently observed (6). By dikaryotic-monokaryotic pairings, the pear basidiomycete is conspecific with this alfalfa *Coprinus*.

Isolates are maintained by the Agriculture Canada Research Station, Lethbridge, Alberta, and the Mid-Columbia Experiment Station, Oregon State University, Hood River, OR.

Basidiocarps of the *Coprinus* sp. on alfalfa are small (4.0–7.0 cm tall and 0.7–1.2 cm wide) and evanescent. They appear usually in the fall under very moist conditions, develop within 24 hr, and quickly begin to autolyse after reaching maturity. This may explain the failure to observe sporulating stages in the life cycle of the pear *Coprinus*.

Hanna (2) reported the production of sclerotia in cultures by the *Coprinus* identified then as *C. urticicola* from wheat and nettles at 10 C. Production of sclerotial patches by the pear *Coprinus* supports this observation. These structures may be significant in survival of the fungus on crating materials used in cold storage of fruit.

A single, preharvest ziram application significantly reduced *Coprinus* infection of fruit both in the orchard and in cold storage, and use of chlorine and benomyl in commercial packinghouses may provide additional control. Additional studies in progress involve the effect of environmental factors on *Coprinus* sporulation and infection in the orchard in order to improve the timing of preharvest fungicide applications.

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