# Interaction and Control of Alternaria Stem Decay and Blue Mold in d'Anjou Pears

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**ABSTRACT**

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Decay of stems of d'Anjou pears by *Alternaria alternata* is found in fruit treated with benomyl postharvest drench for control of *Penicillium expansum* and subsequently held in storage for prolonged periods. Benomyl favors the incidence of *A. alternata*, a slower growing and less competitive fungus than *P. expansum*, at the osmotic potential (−22 bars) and temperature (near 0 C) conditions of pears in storage. Prochloraz, chlorothalonil, iprodine, and triadimenol, in combination with benomyl, were effective in controlling Alternaria stem decay and blue mold on fruit treated and stored for 6 or 7 mo in cold storage. The most effective control of both Alternaria stem decay and blue mold was obtained with 1,000 μg/ml prochloraz combined with 500 μg/ml benomyl. Reduction of linear growth of *A. alternata* on potato-dextrose agar amended with prochloraz was not a useful method for determining the most effective concentration for disease control; 1 μg/ml inhibited mycelial growth of *Alternaria* but 1,000 μg/ml was required for disease control.

An increase in the incidence of stem (pedicel) decay of d'Anjou pears (*Pyrus communis* L.) incited by *Alternaria alternata* (Fries) Keissler (=*A. tenuis* Nees) is associated with use of postharvest drenches of benomyl for controlling blue mold rot of pears incited by *Penicillium expansum* (Link) Thom (1,12,13,15). Fruit rots arising from pear stem infections with *P. expansum* and *Botrytis cinerea* have caused losses (9,11). Benzimidazoles have virtually eliminated these losses. Alternaria stem decay rarely causes extensive damage to the pear flesh and is primarily a "cosmetic" disorder that reduces the market quality of the fruit. The disease is characterized by a slow-moving black lesion extending from the abscission zone toward the flesh of the pear (Fig. 1). Fruit receivers and commercial fruit buyers are suspicious of stem discolorations such as those caused by *Alternaria* because they resemble stem disease incited by *P. expansum* and *Mucor* spp. On the other hand, blue mold, which moves rapidly down the stem, causes damage (Fig. 2) to the flesh of pears. Benomyl and thiabendazole have little effect on *A. alternata* (1−3,6,10,12,15) but significantly reduce infection by *P. expansum* on d'Anjou pears. An increase in incidence of *Alternaria citri* follows thiabendazole treatments to control *P. digitatum* Sacc. and *P. italicum* Wehmer in stored citrus fruits (3,10).

The purpose of this study was 1) to determine why incidence of *A. alternata* increases when benzimidazole is used to control blue mold, 2) to screen fungicides for control of stem decay caused by *Alternaria* and *Penicillium* on pears and to find the best combination of fungicides for controlling stem decay, and 3) to find the "threshold" concentration of fungicide needed to control d'Anjou pear stem decay.

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## MATERIALS AND METHODS

**Measuring water potentials of pear pedicels.** Four healthy pears and two pears with obvious infection by blue mold were selected from cold storage (3 C) after 3 mo. The stems were split longitudinally and a 7-mm-diameter filter paper disk was inserted in the slit. The stem with disk was crushed in a double-roller (home-made) press. Immediately, the juice-laden disk was placed in Wescor C-51 sample chamber connected to a Wescor HR-33 dewpoint microvolt meter (7) that had been calibrated previously with KCl solutions.

**Growth of *P. expansum* and *A. alternata* at various osmotic water potentials.** A basal medium was prepared as follows: 0.75 g $Na_2PO_4$; 0.75 g $KH_2PO_4$; 0.21 g $MgSO_4$; 0.10 g NaCl; 0.40 g $NH_4NO_3$; 1.8 g glucose; 0.1 g yeast extract; 1 g malt extract; and 15 g agar in 1 L distilled water. KCl (6−148 g) was added to individual aliquots (5) to make an osmotic media series (−6, −14, −28.1, −41, −55.8, −72.7, and −91 bars, respectively). Agar blocks 5 mm in diameter were cut from 2-wk-old cultures of *A. alternata* and *P. expansum* and placed in the center of each plate. Each treatment was replicated three times. The plates were stored in sealed polyethylene bags at 11, 3, and 0.6 C. Colony diameters were measured after 19 days.

**Inoculum source and preparation.** Conidia of *A. alternata* and *P. expansum* were collected from pears with stem decay symptoms. Single-spore isolates were obtained by removing a small quantity of spores from the infected fruit with a surface-sterilized flattened needle and transferring them to a 5-ml sterile

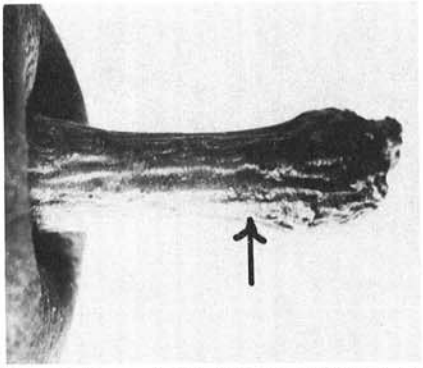

**Fig. 1.** Stem of d'Anjou pear infected by *Alternaria alternata*. Arrow points to the leading edge of the lesion.

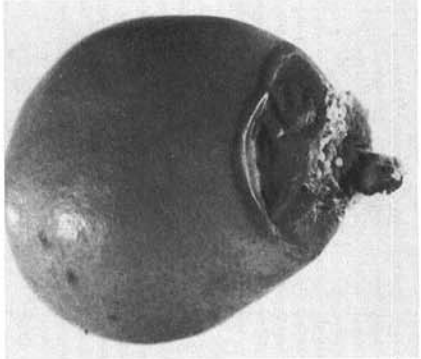

**Fig. 2.** Typical neckrot symptom in d'Anjou pears produced by *Penicillium expansum* as a result of pedicel infection.

water blank. The suspension was dispersed over a 2% water agar plate, excess water was decanted, and the plates were incubated at about 25 C on a laboratory bench for 12 hr. Single-germinated conidia were removed and transferred to one-fifth-strength potato-dextrose agar (PDA) made with 40 g potatoes, 3 g dextrose, and 20 g agar per liter of water. Heavy sporulation occurred in 14 days. Conidia were removed in a water suspension with a drop of Tween 20 (polyoxyethylene sorbitan monolaurate) to improve spore dispersion, and the concentration of conidia in the inoculum was determined with a hemacytometer. The concentration of conidia of both fungi was adjusted to approximate equality (27,000–34,000 conidia/ml). The vials containing the two separate aqueous conidial suspensions were packed in crushed ice to slow germination and protect the spores from heat during transit to the orchard.

**Observation of *Alternaria* conidia on pear stems.** With a razor blade, pedicels were removed from non-fungicide-treated pears in the orchard and immediately fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 wk. The pedicels were postfixed in 2% osmium tetroxide for 2 hr, dehydrated in a graded ethanol series, and passed through a series of ethanol and Freon 113 to pure Freon 113. They were then critical-point dried in a Bomar critical-point drying apparatus (8). The dried specimens were mounted on stubs, coated with a 15-nm layer of gold, and examined in an ETEC Autoscan scanning electron microscope.

**Inoculating pear pedicels.** All fruits used in each experiment were taken from a single tree at the Tree Fruit Research Center, Wenatchee, WA. They were removed by carefully breaking the abscission layer and packed in polyethylene-lined boxes. Care was taken to minimize desiccation of the abscission layers of the pear stems. Within 2 hr of fruit harvest, the stems were dipped in a swirling conidial suspension on a magnetic stirrer. The inoculated fruits were dried stem-end down in the shade.

**Fungicide treatments.** d'Anjou pears were treated for 3 min in a 1,000-$\mu$g/ml solution of benomyl 50W in a 19-L galvanized metal washtub. The benomyl-treated fruit was air-dried.

The following additional treatments were applied to the benomyl-treated and untreated fruit at a rate of 500 $\mu$g/ml (active ingredient): triademenol 14F, prochloraz (bfn 8077) 40F, cholorothalonil (Daconil 2878) 75WP, anilazine (Dyrene) 40F, dichlone 50WP, captafol (Difolatan) 40F, and iprodione (RP 26019) 50WP. After the fruits had dried, they were placed in paper pulp trays and packed in polyethylene-lined fiberboard cartons. They were transferred to refrigerated storage at −0.5 C (±0.25 C) within 6 hr.

**Assessing disease.** After 7 mo, the pears were removed from cold storage and the stems were cut from each pear and measured. The length of the discolored tissue from the abscission layer to the leading edge of fungal growth was also determined.

To isolate the invading organism, small portions (4–5 mm) of diseased tissue from the leading edge of the lesions were dissected and surface-disinfested in 4% NaOCl and 50% ethanol for 10 sec. The tissue was rinsed in running tap water, allowed to stand in sterile distilled water for 15 min, and placed on paper towels in a laminar flow hood until dry. The tissue was transferred to PDA (4 g Difco PDA mixed with 15 g agar in 1 L water) plates and incubated at 11 C for 14 days.

**In vitro inhibition of *Alternaria*.** Prochloraz was added (14) to PDA basal medium in the following concentrations: 1,000, 500, 50, 10, 5, 1, 0.1, 0.01, and 0.001 $\mu$g/ml and a water control. Eighteeen-hour-old germlings of *A. alternata* growing on water agar blocks were transferred to individual fungicide-amended agar plates and to a water control. The petri plates, placed in polyethylene bags to reduce moisture loss, were incubated in the laboratory at 24 C for 6 days. Colony diameters were measured with a ruler.

**In vivo inhibition of *Alternaria*.** d'Anjou pears were immersed in a 500-$\mu$g/ml benomyl suspension for 3 min and allowed to dry in the shade. A prochloraz treatment was then applied as 3-min immersions in the following concentrations: 1,000, 100, 10, 1, 0.1, and 0.01 $\mu$g/ml and a water control. After 6 mo at −0.5 C, the linear growth of stem discoloration was measured with a ruler and fungal isolations were made.

## RESULTS

**Observation of *Alternaria* conidia on pear stems.** Free conidia were found on the epidermis of healthy pear pedicels adjacent to the abscission layer (Fig. 3B). The deep furrow created by the fleshy stems of d'Anjou pears (Fig. 3A) accumulated spores, debris, and a place to protect mites.

**Growth of stem-rotting fungi.** At 11, 3, and 0.6 C under osmotic water potentials from −6.0 to −91 bars, *P. expansum* grows faster than *A. alternata* (Fig. 4). Healthy green pear pedicels had osmotic water potentials of −22 bars when measured by thermocouple psychrometry. Therefore, at 11, 3, and 0.6 C, it can be assumed that *P. expansum* grows faster than *A. alternata* (in a pear stem).

**Controlling stem decay with fungicides.** All seven fungicides at some concentration, or in some combination with

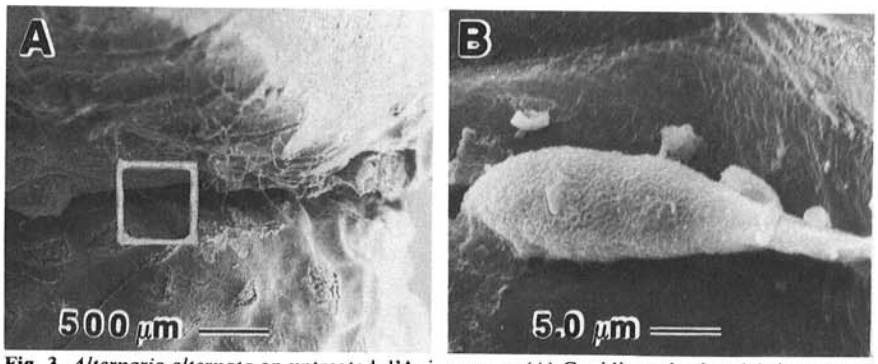


**Fig. 3.** *Alternaria alternata* on untreated d'Anjou pears. **(A)** Conidia and other debris collect in the deep furrow created by the abscission zone of d'Anjou pears. **(B)** Conidium observed in the area delineated by the square in (A).

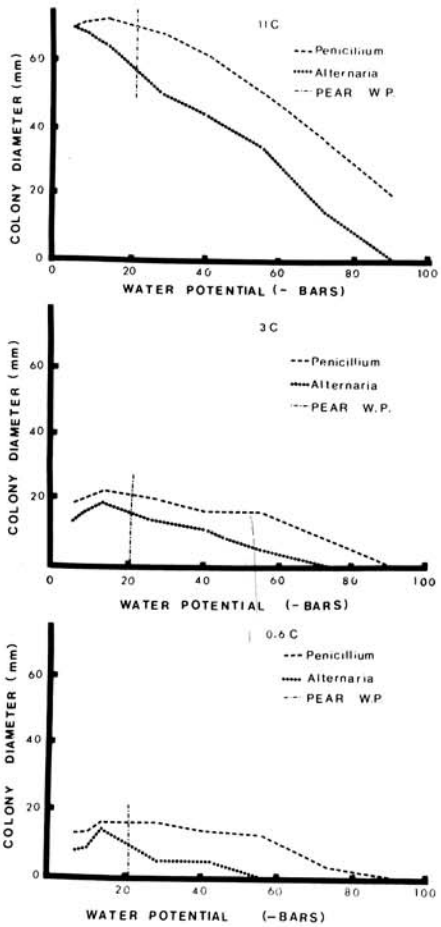


**Fig. 4.** Growth of *Penicillium expansum* and *Alternaria alternata* at 11, 3, and 0.6 C on PDA amended with KCl to achieve osmotic water potentials varying from −6 to −91 bars.

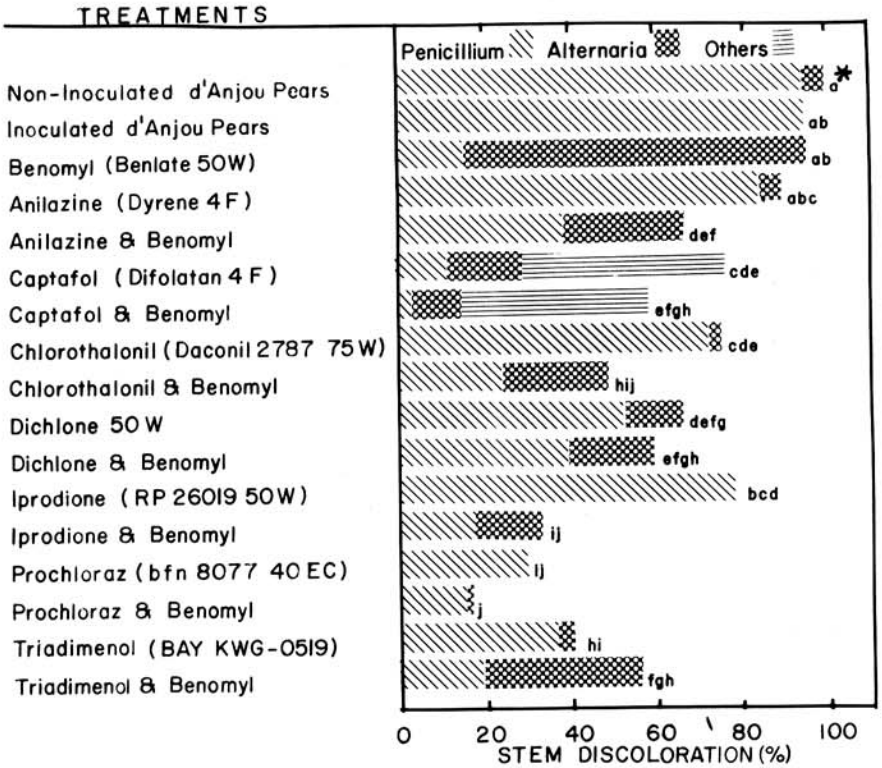


**Fig. 5.** Effect of various fungicide treatments in controlling d'Anjou pear pedicel discoloration as a result of *Penicillium expansum*, *Alternaria alternata*, and other fungi after storage 7 mo at −0.5 C. Means in a bar not followed by the same letter are significantly different at the 5% level according to Duncan's multiple range test.

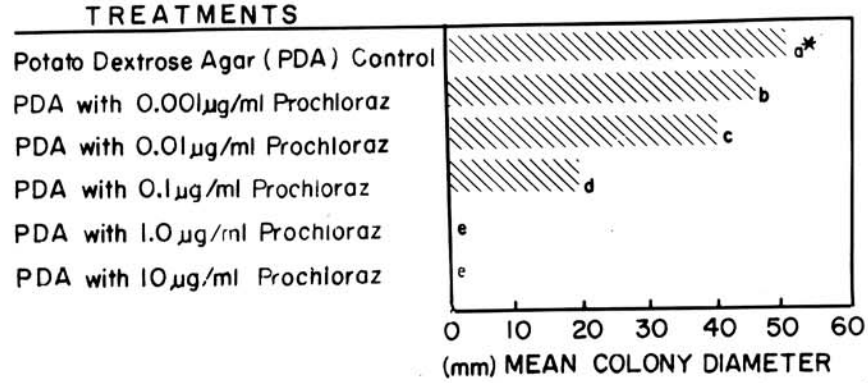


**Fig. 6.** In vitro effect of various concentrations of prochloraz-amended PDA on the linear growth of *Alternaria alternata*. Means in a bar not followed by the same letter are significantly different at the 5% level according to Duncan's multiple range test.

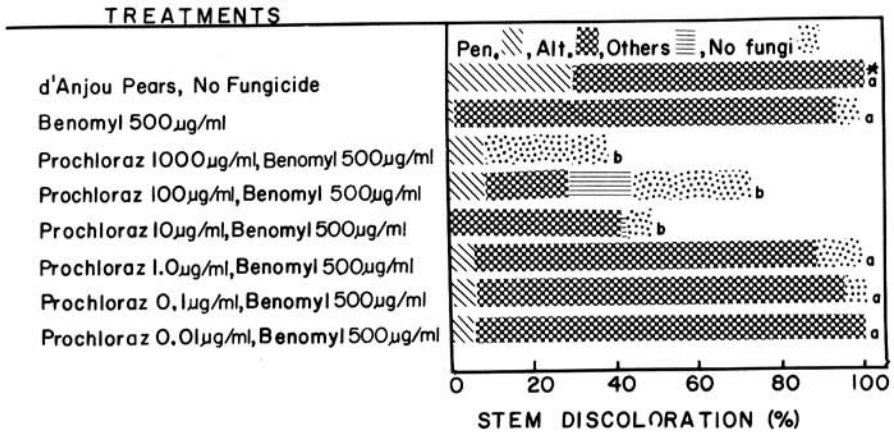


**Fig. 7.** Effect of various concentrations of prochloraz (bfn 8077) on d'Anjou pears after storage 7 mo at −0.5 C. Means in a bar not followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

other fungicides, controlled Alternaria stem decay and blue mold except benomyl and anilazine (Fig. 5). The best results were obtained with a combination of benomyl and prochloraz. This mixture reduced stem decay from 100 to 17.4% (6). Prochloraz alone reduced infection to 30.8% and reduced blue mold as well. Chlorothalonil, iprodione, and triadimenol combined with benomyl substantially reduced blue mold and Alternaria stem decay. Benomyl alone increased Alternaria stem decay and simultaneously reduced blue mold.

Captafol and dichlone were least effective in controlling discoloration associated with infection by *A. alternata* and *P. expansum*. They did reduce Alternaria stem decay, however, when compared with benomyl alone.

Prochloraz-amended PDA was not useful for establishing threshold concentrations for control of d'Anjou pear stem decay. Growth of *A. alternata* (Fig. 6) was completely inhibited at a concentration of 1 µg/ml prochloraz and some inhibition was evident as low as 0.001 µg/ml.

Based on the in vitro information, a dilution series was prepared to test prochloraz for controlling stem discoloration (Fig. 7). Substantial reduction was obtained with as little as 10 µg/ml prochloraz, but complete inhibition of *A. alternata* occurred only at 1,000 µg/ml. Concentrations greater than 0.1 µg/ml resulted in some stems producing a nonfungal browning. When these stems were surface-disinfected and placed on PDA, no fungi or bacteria grew from the pieces. This study produced no evidence of the nature of this nonfungal stem discoloration associated with prochloraz fungicide treatments.

## DISCUSSION

Incidence of Alternaria stem decay tends to increase when benzimidazole fungicides are used to control blue mold rot. This apparent increase may be the result of reduced competition by *P. expansum*. *P. expansum* grew faster than *A. alternata* under all water potentials and temperatures studied. This faster growth may provide a competitive advantage over *A. alternata*. Use of a fungicide like benomyl, which controls a fast-growing fungus (4), may favor the growth of a slower-growing, more tolerant fungus like *A. alternata*.

Alternaria stem decay will continue to be a problem in d'Anjou pears until a fungicide or combination of fungicides is used commercially that is effective against both fungi. In the meantime, registration of fungicides like prochloraz (1,000 µg/ml) to be combined with benomyl (500 µg/ml) would facilitate control of stem decay and increase the market value of d'Anjou pears stored for extended periods of time.

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