Cryptococcus humicola Inhibits Development of Lesions in ‘Golden Delicious’ Apples

Jeffrey A. Anderson

Department of Horticulture and Landscape Architecture, Oklahoma State University, Stillwater, OK 74078-6027

Alexander B. Filonow

Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078

Helen S. Vishniac

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078

Additional index words. gray mold, *Malus ×domestica*, biological control, antagonist, fungus, *Sporobolomyces roseus*, *Botrytis cinerea*

Abstract. One strategy to reduce postharvest losses of fruits to pathogens is to introduce organisms with biological control capabilities. Our objective was to determine the effectiveness of two yeast isolates in inhibiting lesion development caused by *Botrytis cinerea* (Bc) on freshly harvested apples differing in maturity. ‘Golden Delicious’ apples were harvested on 29 Aug., 23 Sept., and 10 Oct. 1995. Apples receiving the seven treatments [control, wound, *Cryptococcus humicola* (Ch), *Sporobolomyces roseus* (Sr), Bc, Ch + Bc, Sr + Bc] were placed in plastic boxes with damp paper towels. Each day for 7 days, ethylene production and lesion diameter at the wound were recorded. Ethylene production was not affected by treatment, and increased with later harvest date. Lesion diameter on apples treated with Bc was smaller on the first harvest than on the second and third harvests. Sr provided partial control on the second and third harvests, and Ch completely inhibited lesion development except for day 7 of the third harvest.

Up to 30% of fruits and vegetables may be lost annually to postharvest disease worldwide (Sams, 1994). Biological control of postharvest pathogens avoids many of the shortcomings of chemical fungicides, including potentially harmful residues on food and emergence of resistant strains of pathogens. Many different organisms, including yeasts and bacteria, have reduced the incidence or severity of fungal lesions (Droby et al., 1989; Mercier and Wilson, 1995; Wisniewski and Wilson, 1992). Previously, we surveyed 28 yeast isolates from diverse sources and identified several that effectively reduced the incidence of fungal decay on apples (*Malus ×domestica* Borkh.) purchased from a supermarket (Filonow et al., 1996). Our objective here was to determine the effectiveness of two yeast isolates in inhibiting lesion development caused by *Botrytis cinerea* on freshly harvested apples differing in maturity. This study expanded our previous findings to include apples that had not been

treated with a postharvest fungicide and had not undergone physiological changes associated with storage.

Materials and Methods

Cryptococcus humicola [(Daszewska) Golubev] NRRL Y1266 (Ch) was obtained from the U.S. Dept. of Agriculture Northern Regional Research Laboratory collection (Peoria, Ill.). *Sporobolomyces roseus* (Kluyver & Niel) FS-43-238 (Sr) and *Botrytis cinerea* F-J-4 (Bc) were provided by W. Janisiewicz (U.S. Dept. of Agriculture, Agricultural Research Service, Kearneysville, W.Va.). ‘Golden Delicious’ apples were harvested from a commercial orchard in Porter, Okla., on 29 Aug., 23 Sept., and 10 Oct. 1995. On each date, 45 uniform fruits were selected from a sample of several hundred apples based on appearance and ethylene evolution rate. Ethylene emis-

sions were measured as described previously (Anderson and Peters, 1995), except that fruits were held in 3900-mL glass jars fitted with septa for 2 h before removing 1 mL of headspace samples. Ten apples were destructively sampled for firmness measurements. The puncture resistance force was measured on opposite sides, using an Effegi penetrometer (Effegi, Alfonsive, Italy) fitted with an 8-mm probe.

On each harvest date, five apples were assigned randomly to each of seven treatments. All apples, except controls, were punctured (2 mm in diameter × 4 mm deep) at the equator with a heat-sterilized, tapered glass rod. Wounds received 20 µL of a suspension (5×10^6 cells per mL) of a biocontrol yeast (either Ch or Sr) or sterile water. Within 30 min after biocontrol treatment, wounds received 20 µL of sterile water or 2×10^4 conidia per mL of the pathogen (Bc). Apples (control, wound, Ch, Sr, Bc, Ch + Bc, Sr + Bc) were randomly distributed into two 26.5-L plastic boxes with damp paper towels to maintain a moist environment. Lesion diameter at the wound was recorded each day for 7 d. Each day following lesion measurements, apples were placed in glass jars for 2 h to measure ethylene production.

Data were transformed [$\log(\text{value} + 0.1)$] due to nonhomogeneity of variance based on Bartlett's test (Gomez and Gomez, 1984). Firmness data were analyzed in a one-way analysis of variance (ANOVA). Lesion diameter and ethylene data were initially analyzed as a split plot, with treatment as the main plot and day of observation as the subplot, using the general linear models procedure (SAS, Cary, N.C.). Since only lesion data for observations made on the 7th day are shown, data were reanalyzed in a one-way ANOVA by treatment. Harvest date means were separated where appropriate by Duncan's multiple range test at $P \leq 0.05$.

Results and Discussion

Firmness measurements suggested that apples from the first two harvests were immature, and that apples harvested on 10 Oct. were past optimum maturity (Table 1). Ethylene production did not differ among treatments, so data were averaged over treatments within each harvest. No ethylene was detected initially from apples harvested 29 Aug. (data not shown). Evolution rates increased to ≈ 0.7 nmol·kg⁻¹·s⁻¹ 7 d after treatment, indicating that some ripening occurred during the observation period. Apples harvested 23 Sept. had

Received for publication 12 Nov. 1996. Accepted for publication 6 May 1997. We thank Kent Livesay for donating apples, Glenn Drown for technical assistance, and Mark Payton for statistical advice. Approved for publication by the Director, Oklahoma Agricultural Experiment Station. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 1. Biological control of gray mold on apples by yeasts. Apples were evaluated 7 d after artificial wounds were inoculated with a biocontrol yeast (Ch or Sr) and/or a fungal pathogen (Bc). The wound treatment received only sterile water. The number of apples with lesions is indicated in parentheses (n = 5).

Harvest date, 1995	Firmness at harvest (N)	Lesion diameter (mm)						
		Control	Bc	Ch + Bc	Sr + Bc	Wound	Ch	Sr
29 Aug.	69 a ²	0	18.8 (5) a	0	19.8 (5) a	0	0	0
23 Sept.	62 b	0	36.0 (5) b	0	28.0 (2) b	0	0	0
10 Oct.	20 c	0	40.6 (5) b	14.0 (1)	21.0 (4) a	15.5 (2)	0	12.0 (1)

²Mean separation (when appropriate) within columns by Duncan's multiple range test at $P \leq 0.05$.

higher ethylene evolution rates than those from the first harvest, with values increasing from ≈ 0.7 to $1.6 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{s}^{-1}$ from 1 to 7 d after treatment, respectively. Ethylene production was $\approx 2 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{s}^{-1}$ throughout the observation period for apples harvested 10 Oct.

Lesions became visible 3 to 4 d after inoculation. Lesions that developed on apples treated only with Bc were smaller on days 5 through 7 after treatment following the first harvest, in comparison with the 23 Sept. and 10 Oct. harvests. No significant reduction in lesion diameter was observed with Sr co-inoculated with Bc on apples harvested 29 Aug. (Table 1). No lesions were observed in treatments other than Bc and Sr plus Bc for fruits harvested 29 Aug., indicating complete control by isolate Ch. Partial and complete control were observed with Sr and Ch, respectively, for fruit harvested 23 Sept.; Sr reduced both the proportion of apples with lesions and the mean lesion diameter. Lesion development on noninoculated apples harvested 10 Oct. may have resulted from increased inoculum densities of pathogens in the orchard or reduced resistance of the apple fruit. Spotts (1985) reported that 'Anjou' and 'Bartlett' pear (*Pyrus communis*

L.) fruit became more susceptible to Bc as they matured. No attempt was made to identify the pathogens in noninoculated apples. Lesions were also observed on sites remote from the wound on some fruits harvested late. While Sr still reduced both the proportion of apples with lesions and the mean lesion diameter, this third harvest was the only one in which Ch failed to provide complete protection from gray mold infection.

Ch was highly effective in reducing lesion development in apples from all three harvests, whereas Sr was effective only in the second and third harvests. Sr had been shown to be an effective biocontrol agent against gray mold of apple at inoculum concentrations similar to ours (Janisiewicz et al., 1994), so the reduced effectiveness of Sr in our study may have been due to differences in apple quality or experimental technique.

Literature Cited

- Anderson, J.A. and D.C. Peters. 1995. Ethylene biosynthesis and action inhibitors do not prevent injury to wheat seedlings infested with *Schizaphis graminum*. *Environ. Entomol.* 24:1644–1649.
- Droby, S., E. Chalutz, C.L. Wilson, and M. Wisniewski. 1989. Characterization of the biocontrol activity of *Debaryomyces hansenii* in the control of *Penicillium digitatum* on grapefruit. *Can. J. Microbiol.* 35:794–800.
- Filonow, A.B., H.S. Vishniac, J.A. Anderson, and W.J. Janisiewicz. 1996. Biological control of *Botrytis cinerea* in apple by yeasts from various habitats and their putative mechanisms of antagonism. *Biol. Control* 7:212–220.
- Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. Wiley, New York.
- Janisiewicz, W.J., D.L. Peterson, and R. Bors. 1994. Control of storage decay of apples with *Sporobolomyces roseus*. *Plant Dis.* 78:446–470.
- Mercier, J. and C.L. Wilson. 1995. Effect of wound moisture on the biocontrol by *Candida oleophila* of gray mold rot (*Botrytis cinerea*) of apple. *Postharvest Biol. Technol.* 6:9–15.
- Sams, C.E. 1994. Management of postharvest disease resistance in horticultural crops: Introduction to the colloquium. *HortScience* 29:746–747.
- Spotts, R.A. 1985. Effect of preharvest pear fruit maturity on decay resistance. *Plant Dis.* 69:388–390.
- Wisniewski, M. and C.L. Wilson. 1992. Biological control of diseases of fruits and vegetables, recent advances. *HortScience* 27:49–98.