Integration of Cultural Methods with Yeast Treatment for Control of Postharvest Fruit Decay in Pear

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ABSTRACT

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Pear fruit from trees managed for high or low fruit nitrogen and calcium content were harvested either early or late in the normal maturity range, wounded, and then treated with either water or yeast suspensions (*Cryptococcus laurentii, C. flavus*, or *C. laurentii* + one-tenth the label rate of thiabendazole [Mertect 340F]). Prior to storage, wounds were inoculated with spore suspensions of *Penicillium expansum*, causal agent of blue mold, or *Phialophora malorum*, causal agent of side rot. Fruit were stored 2–3 mo in air or controlled atmosphere (2% O_2, 0.6% CO_2) at 0 C. Early harvest, low fruit N, high fruit Ca, yeast or yeast + fungicide treatment, and controlled atmosphere storage all reduced severity of blue mold and side rot. When optimum levels of each treatment were combined, blue mold severity was reduced by about 95% in 1991, 61% in 1992, and 98% in 1993. In 1991, side rot was completely controlled in all treatments that included *C. laurentii* + early harvest or *C. laurentii* + high fruit Ca. In 1992, no side rot developed when treatments included *C. laurentii* + early harvest + high fruit Ca. Without yeast treatment, the combination of early harvest, low fruit N, and high fruit Ca in air storage reduced severity of blue mold by 64% and reduced side rot by 97% in 1991. More disease developed in pears treated with *C. flavus* than in those treated with *C. laurentii*.

Additional keywords: biological control, integrated control, *Pyrus communis*

Postharvest fungal decay of pear (*Pyrus communis* L.) fruit causes substantial economic loss for the fruit industry of the Pacific Northwest (8). Although postharvest fungicide treatments can reduce decay, products currently registered in the United States for pear are not effective against all important fungal pathogen species (22). In addition, resistance to postharvest fungicides has been documented in key pathogen species (1), and in some markets the sale of fruit with detectable residues of postharvest fungicides is prohibited (22). Consequently, other methods to control decay are needed that either complement or replace fungicide treatments.

Treatments that alter fruit nutrient status can influence the susceptibility of pome fruit to postharvest decay. Calcium chloride sprays during the growing season increase fruit calcium and reduce severity of side rot caused by *Phialophora malorum* (M.N. Kidd & A. Beaumont) McColloch in pear (17). Postharvest pressure infiltration of apple fruit with calcium choride reduces severity of blue mold caused by *Penicillium expansum* Link in apple (4). Lower fruit nitrogen content is associated with reduced severity of blue mold decay in pear, and management of pear trees for low fruit nitrogen combined with summer calcium chloride sprays further reduces decay severity (18). Fruit nitrogen content in pear is influenced by timing of fertilizer application to the soil (12). Fertilizer applied approximately 1 mo prior to harvest results in a minimum of fertilizer N partitioned to fruit, whereas pre- or postbloom applications result in a relatively high proportion of fertilizer N partitioned to fruit (12).

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Fruit maturity affects susceptibility to postharvest decay (2). The primary indicator of maturity in pear fruit is firmness, which is measured with a penetrometer (23). A range of firmness values appropriate for commercial harvest is established for each pear cultivar in each production region. In the Rogue River Valley of Oregon, harvest of Bosc pears begins at a firmness of approximately 71 newtons (1 newton = 0.2248 lb) and should be completed by 53 newtons. The severity of several postharvest diseases of pear increases as fruit approach maturity (15) and as harvest is delayed within the range of harvestable maturity (2).

Storage atmospheres of reduced O_2 and elevated CO_2 inhibit senescence of pome fruit in long-term storage and can reduce severity of postharvest fungal decay (3). However, levels of CO_2 that substantially reduce fungal activity can cause physiological disorders in fruit (14).

Biological control of postharvest fungal decay in pome fruit by several bacteria (5,6) and yeast species (9,10) has been demonstrated experimentally. The yeast *Cryptococcus laurentii* (Kuff.) C.E. Skinner is able to colonize wounds in pear fruit under conditions of low temperature (0 C), ambient or reduced O_2, and ambient or elevated CO_2 (11,13). Control in pear of Mucor rot caused by

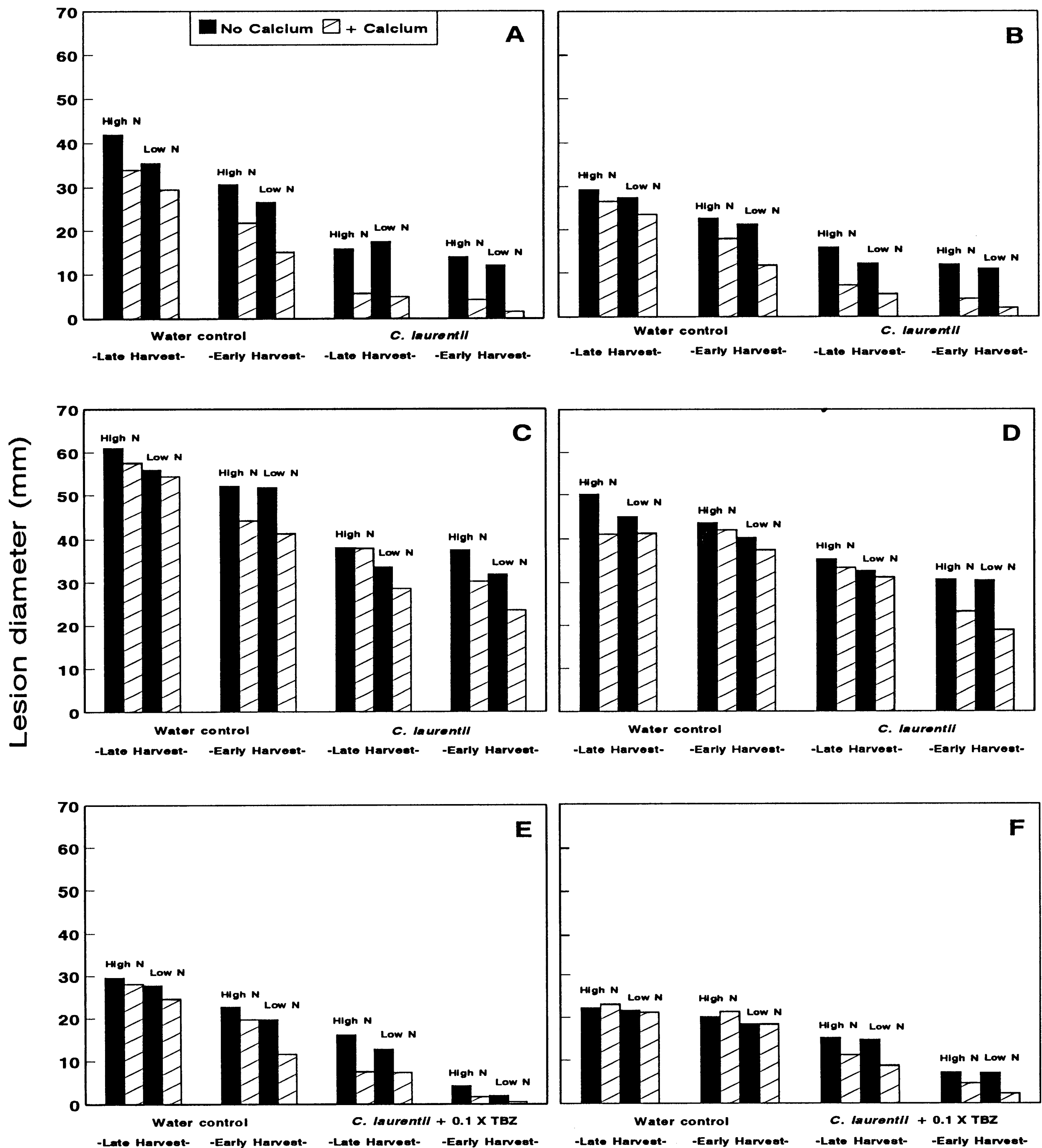

Fig. 1. Severity of blue mold decay, caused by *Penicillium expansum*, in Bosc pear fruit from trees managed for high or low fruit nitrogen and calcium, harvested early or late in the maturity range, and treated postharvest with water or the yeast *Cryptococcus laurentii*. Inoculated fruit were stored 2–3 mo at 0 C in **(A, C, E)** air or **(B, D, F)** controlled atmosphere (2% O_2, 0.6% CO_2). Data are from the **(A, B)** 1991, **(C, D)** 1992, and **(E, F)** 1993 seasons. In 1993, *C. laurentii* was combined with thiabendazole (TBZ) at one-tenth the label rate (56.9 mg a.i./L).

Mucor piriformis E. Fischer (10) and gray mold caused by *Botrytis cinerea* Pers.:Fr. (9) has been demonstrated.

The objective of this study was to evaluate the postharvest disease severity in pear fruit varying in nitrogen and calcium status, maturity at harvest, storage atmosphere, and postharvest yeast treatment as factors composing integrated programs. Levels of these factors were tested factorially for control of blue mold, caused by *P. expansum*, a ubiquitous and aggressive pathogen (8), and side rot, caused by *Phialophora malorum*, a disease currently prevalent only in southern Oregon and a relatively weak pathogen (20). An abstract of a portion of this work has been published (19).

MATERIALS AND METHODS

Nitrogen and calcium management. A pear orchard (cv. Bosc on *P. betulaefolia* Bunge and *P. calleryana* Decne. rootstocks) of approximately 1.4 ha was used for field treatments. Trees were spaced at 2.5 × 4.2 m and were 12–15 yr old during the study. The orchard was divided into 10 40-tree plots (four rows × 10 trees) for N management. Five randomly selected plots were not fertilized for 2 yr, then urea at 45 kg/ha was applied to the soil annually 1 mo prior to harvest. We previously demonstrated that fertilizer application 1 mo prior to harvest resulted in minimum fruit N content (12). Accordingly, these were considered low fruit N treatments. The other five plots received annual applications of 90 kg/ha of urea to the soil in the tree rows 2 wk after full bloom and were considered high fruit N treatments. Within each N management plot, eight trees near the center of the plot were selected randomly for calcium treatments. Four of the trees in each plot were sprayed to runoff with $CaCl_2$ solutions at 6 g/L of calcium. Calcium applications began in early July and were repeated every 2 wk for a total of three sprays annually. Four trees not sprayed with $CaCl_2$ in each plot were identified as untreated controls (low fruit Ca). The effect of $CaCl_2$ sprays on Ca content and decay resistance of pear fruit has been described previously (17).

Harvest maturity. Fruit firmness was measured weekly as the normal harvest period approached each year. Firmness was measured on a Magness-Taylor penetrometer with a 6-mm tip. When average firmness values reached approximately 71 newtons, 130 fruit were harvested from each group of $CaCl_2$-treated and untreated trees within each replicate N management plot (early harvest treatment). These fruit were stored in polyethylene-lined fiberboard cartons at 0 C. When average firmness values reached approximately 55 newtons, another 130 fruit were harvested from each group of $CaCl_2$-treated and untreated trees in each N management plot and stored similarly (late harvest treatment). Ten fruit from each replicate of each harvest treatment were tested for firmness after harvest.

Postharvest yeast treatment. The yeasts *Cryptococcus flavus* (Saito) Phaff & Fell and *C. laurentii* were originally isolated from the surface of apples (9,13). To prepare inoculum for postharvest treatments, each yeast was grown in petri dishes on agar consisting of 20 g of Difco nutrient agar, 4 g of yeast extract, and 1.5 g of dextrose per liter of distilled water. After 3–4 days of incubation at 24 C, suspensions of each species were made by scraping colonies from the agar surfaces into tap water. With the aid of a spectrophotometer, yeast concentrations were adjusted to 1×10^8 cells per milliliter (2% transmittance at 595 nm) (9).

Fruit from each replicate of each N × $CaCl_2$ × harvest maturity treatment were surface-disinfested for 5 min in a 0.5% NaOCl solution. They were then rinsed in fresh water and placed on fiberboard fruit trays on a laminar-flow sterile bench. Each fruit was wounded in five locations with the end of a sterile steel finishing nail. Wounds were approximately 3 mm deep and 6 mm in diameter. In 1991, 10 fruit (50 wounds) of each replicate were inoculated with suspensions of *C. laurentii*, 10 with *C. flavus*, and 10 with sterile water as a control. In 1992, only *C. laurentii* and water treatments were applied. In 1993, *C. laurentii* was combined with the fungicide thiabendazole (Mertect 340F) at 56.9 mg a.i./L, one-tenth the label rate for pears (22). A micropipette was used to place 50 μl of yeast suspension, yeast suspension + fungicide, or water in each wound.

Following yeast treatment, all wounds were inoculated with 40 μl of a conidial suspension of *P. expansum* (1×10^4 conidia per milliliter). An identical set of treatments was inoculated with a conidial suspension of *Phialophora malorum* (1×10^4 conidia per milliliter). *Phialophora malorum* was not included in experiments in 1993. Cultures of both pathogens were originally isolated from decay lesions on pear fruit in Medford, Oregon. Conidial suspensions were prepared by washing the surfaces of 3-wk-old colonies growing on Difco potato-dextrose agar with distilled water and adjusting spore concentration with a hemacytometer.

Storage atmosphere treatment. Trays of inoculated fruit were loosely covered with perforated polyethylene bags, stacked in fiberboard boxes, and placed in a commercial controlled atmosphere storage maintained at 2% O_2 and 0.6% CO_2 at 0 C. An identical set of all treatments described above was stored in ambient air at 0 C.

Blue mold development was measured after 2 mo of storage in 1991 and 1993 and 3 mo of storage in 1992. Side rot development was measured after 3 mo of storage. The diameters of lesions that centered about inoculated wounds were measured with vernier calipers. Data were analyzed by ANOVA using Number Cruncher Statistical System software (J. L. Hintze, Kaysville, UT), with harvest maturity, nitrogen management, calcium treatment, yeast treatment, and storage atmosphere as main factors.

RESULTS

Early harvest, low fruit N, high fruit Ca, yeast treatment, and controlled atmosphere storage all reduced severity

Table 1. Summary of analysis of variance for lesion diameters in Bosc pears treated with cultural and biological methods for control of blue mold caused by *Penicillium expansum* (*Pe*) and side rot caused by *Phialophora malorum* (*Pm*)

		***P* value**				
		1991		**1992**		**1993**
Experimental factor[a]	**df**	***Pe***	***Pm***	***Pe***	***Pm***	***Pe***
Atmosphere (A)	1	<0.001	<0.001	<0.001	<0.001	0.837
Maturity (M)	1	<0.001	<0.001	<0.001	<0.001	<0.001
Yeast treatment (Y)	1	<0.001	<0.001	<0.001	<0.001	<0.001
Fruit nitrogen (N)	1	<0.001	0.239	<0.001	0.002	<0.001
Fruit calcium (C)	1	<0.001	<0.001	<0.001	<0.001	<0.001
A × M	1	0.048	0.009	0.329	<0.001	<0.001
A × Y	1	<0.001	<0.001	<0.001	<0.001	<0.001
A × C	1	0.001	0.583	0.706	0.782	0.024
M × Y	1	<0.001	<0.001	0.344	0.628	0.027
M × C	1	0.011	0.640	0.005	0.293	0.372
Y × N	1	0.003	0.275	0.318	0.081	0.170
Y × C	1	0.007	0.001	0.099	0.054	0.010
A × M × Y	1	0.074	0.001	<0.001	<0.001	0.014
A × Y × C	1	0.655	0.608	0.475	0.044	0.036
M × Y × C	1	0.037	0.499	0.047	<0.001	0.023

[a] Pear fruit from trees managed for high or low fruit nitrogen and calcium content were harvested either early or late in the normal maturity range, wounded, and then treated with cell suspensions of *Cryptococcus laurentii* (10^8 cells per milliliter) or *C. laurentii* + thiabendazole (TBZ) (56.9 mg a.i./L, 1993 only) or with water. Wounds were then inoculated with spore suspensions of either pathogen (10^4 spores per milliliter) and stored for 2–3 mo in air or in controlled atmosphere (2% O_2, 0.6% CO_2) at 0 C.

of blue mold decay (Fig. 1, Table 1) and side rot (Fig. 2, Table 1) in all years of the study. In 1991, the combination of early harvest, low fruit N, high fruit Ca, and *C. laurentii* treatment reduced severity of blue mold decay by about 95% as compared with the reciprocal set of treatments. The same combination reduced blue mold severity by 61% in 1992. In 1993, the combination of early harvest, low fruit N, high fruit Ca, and *C. laurentii* + one-tenth the label rate of thiabendazole reduced blue mold severity by 98%. Side rot was completely controlled in 1991 by all treatments that included *C. laurentii* + early harvest or *C. laurentii* + high fruit Ca. Without yeast treatment, the combination of early harvest, low fruit N, and high fruit Ca in air storage reduced blue mold severity by 33–64% and reduced side rot by 67–97%. Disease severity with *C. flavus* treatment across all other combinations of factors in 1991 was intermediate to that of *C. laurentii* (*data not shown*). Consequently, *C. flavus* was not included in subsequent tests.

In 1991, disease incidence at wounds inoculated with *P. expansum* ranged from 99.6% in high N, low Ca fruit harvested late and treated with water postharvest to 14.8% in low N, high Ca fruit harvested early and treated with *C. laurentii* postharvest. The respective incidences of infected wounds were 100 and 69.6% in 1992 and 100 and 5.6% in 1993. In 1991, disease incidence at wounds inoculated with *Phialophora malorum* ranged from 82.4% in high N, low Ca fruit harvested late and treated with water postharvest to 0% in low N, high Ca fruit harvested early and treated with *C. laurentii* postharvest. In 1992, the respective incidences were 88.4 and 0%.

Calcium chloride treatments increased fruit firmness at harvest significantly ($P \leq 0.05$) (Table 2). The average difference in fruit firmness between calcium-treated and untreated fruit was 2.9 newtons. In 1991, fruit from low N treatments were firmer than fruit from high N treatments at the late harvest but not at the early harvest. This effect was not apparent in 1992 or 1993 (Table 2).

DISCUSSION

These results demonstrate that unrelated cultural and biological methods that influence pear decay susceptibility or disease development can be combined into an integrated program to substantially reduce decay. Although complete disease control was achieved in these experiments only with the relatively weak pathogen *Phialophora malorum*, disease was reduced appreciably with the faster-growing *P. expansum*. In these experiments, the pathogen inoculum was placed directly into relatively large, fresh fruit wounds. Better performance of the integrated program is likely under conditions of lower inoculum concentrations in smaller, partially healed wounds (16,21). The reduction in blue mold severity in pear fruit managed for low N content may be partially negated by very high inoculum concentrations ($\geq 10^6$ per milliliter) (D. Sugar, *unpublished*). In the case of blue mold, all of the factors combined in the integrated program are compatible with thiabendazole, currently registered for postharvest

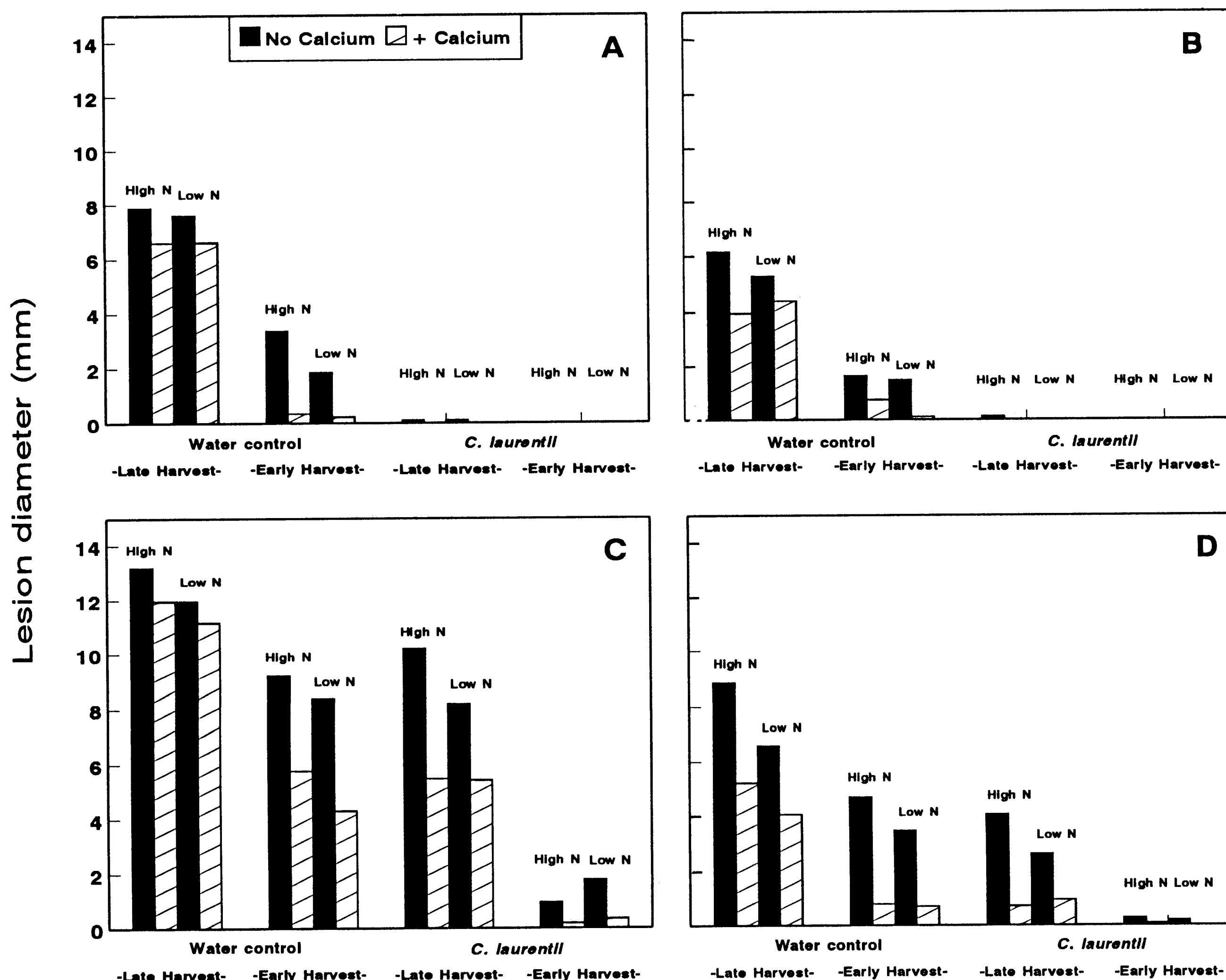

Fig. 2. Severity of side rot, caused by *Phialophora malorum*, in Bosc pear fruit from trees managed for high or low fruit nitrogen and calcium, harvested early or late in the maturity range, and treated postharvest with the yeast *Cryptococcus laurentii*. Inoculated fruit were stored 3 mo at 0 C in **(A, C)** air or **(B, D)** controlled atmosphere (2% O_2, 0.6% CO_2). Data are from the **(A, B)** 1991 and **(C, D)** 1992 seasons.

Table 2. Fruit firmness (in newtons) at harvest of Bosc pears from trees treated for high and low fruit nitrogen (N) and calcium (Ca) and harvested at early and late maturity

	1991				1992				1993			
	Low N		High N		Low N		High N		Low N		High N	
Harvest	No Ca	+Ca	No Ca	+Ca	No ca	+Ca	No Ca	+Ca	No Ca	+Ca	No Ca	+Ca
Early	69.2	71.8	69.2	74.5	72.3	74.5	72.3	74.5	68.7	70.1	66.9	69.2
Late	62.4	65.6	56.2	59.4	58.9	60.7	58.0	61.1	54.0	58.5	53.1	55.8

	P value		
Experimental factor	1991	1992	1993
Maturity (M)	<0.001	<0.001	<0.001
Fruit nitrogen (N)	0.062	0.969	0.172
Fruit calcium (C)	0.008	0.054	0.047
M × N	0.006	0.969	0.580

decay control in pear (11). Thiabendazole does not control infection by *Phialophora malorum* (22).

Several interactions among factors were significant ($P < 0.05$) in their effect on disease severity (Table 1). Differences in disease severity due to harvest maturity were generally less pronounced in fruit stored in controlled atmosphere than in fruit stored in air. The effect of harvest maturity was usually greater in fruit treated postharvest with water than in fruit treated with yeast or yeast + fungicide. Controlled atmosphere storage generally reduced lesion diameters in fruit treated postharvest with water but not in fruit treated with yeast suspensions, except for side rot in 1992.

Reduction of disease severity due to fruit calcium enhancement was often greater in yeast-treated fruit than in water controls. This may be due to decreased pathogen inoculum potential in the presence of the yeast antagonist. Lesion diameter in $CaCl_2$-treated pear fruit is related to inoculum concentration (17). McLaughlin et al (7) reported that biological control of gray mold caused by *B. cinerea* in apple by the yeasts *Candida guilliermondii* (Castellani) Langeron & Guerra and *Kloeckera apiculata* (Reess emend. Klöcker) Janke was enhanced when yeast suspensions included 2% $CaCl_2$.

The larger blue mold lesion diameters found in 1992 as compared with 1991 and 1993 were due in part to the increased length of storage time between inoculation and evaluation. However, side rot lesion diameters also were larger despite harvest firmness values that were similar to or slightly higher than those in 1991 (Table 2). Given that the inoculum density in each year was identical, differences in lesion diameters among years also may have been affected by physiological resistance factors, such as levels of enzyme inhibitors, which may vary from season to season. The effects of culture, atmosphere, and yeast factors and their combinations followed similar patterns in each year (Figs. 1 and 2).

The approach to postharvest pear disease control suggested by this study should have immediate and long-term applications in the fruit industry. Registration of *C. laurentii* for commercial use on pome fruit in the United States is currently pending. All of the other decay-reducing practices evaluated in this study can be used by producers, with or without the inclusion of thiabendazole. If *C. laurentii* becomes available, decay control would be strengthened by its use. As other biocontrol agents become available for use in pear, their compatibility with cultural practices and fungicide treatments will need to be evaluated. Our results may also convince producers to implement practices that in themselves offer only partial control of decay (e.g., fertilizer management for low N fruit) but whose value becomes more apparent as part of an integrated program.

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LITERATURE CITED

1. Bertrand, P. F., and Saulie-Carter, J. 1978. The occurrence of benomyl-tolerant strains of *Penicillium expansum* and *Botrytis cinerea* in the Mid-Columbia region of Oregon and Washington. Plant Dis. Rep. 62:302-305.
2. Boonyakiat, D., Chen, P. M., Spotts, R. A., and Richardson, D. G. 1987. Effect of harvest maturity on decay and post-harvest life of d'Anjou pear. Sci. Hortic. 31:131-139.
3. Chen, P. M., Spotts, R. A., and Mellenthin, W. M. 1981. Stem-end decay and quality of low oxygen stored 'd'Anjou' pears. J. Am. Soc. Hortic. Sci. 106:695-698.
4. Conway, W. S., and Sams, C. E. 1983. Calcium infiltration of Golden Delicious apples and its effect on decay. Phytopathology 73:1068-1071.
5. Janisiewicz, W. J., and Marchi, A. 1992. Control of storage rots on various pear cultivars with a saprophytic strain of *Pseudomonas syringae.* Plant Dis. 76:555-560.
6. Janisiewicz, W. J., and Roitman, J. 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia.* Phytopathology 78:1697-1700.
7. McLaughlin, R. J., Wilson, C. L., Droby, S., Ben-Arie, R., and Chalutz, E. 1992. Biological control of postharvest diseases of grape, peach, and apple with the yeasts *Kloeckera apiculata* and *Candida guilliermondii.* Plant Dis. 76:470-473.
8. Pierson, C. F., Ceponis, M. J., and McColloch, L. P. 1971. Market diseases of apples, pears, and quinces. U.S. Dep. Agric. Handb. 376.
9. Roberts, R. G. 1990. Postharvest biological control of gray mold of apple by *Cryptococcus laurentii.* Phytopathology 80:526-530.
10. Roberts, R. G. 1990. Biological control of Mucor rot of pear by *Cryptococcus laurentii, C. flavus,* and *C. albidus.* (Abstr.) Phytopathology 80:1051.
11. Roberts, R. G. 1991. Characterization of postharvest biological control of deciduous fruit diseases by *Cryptococcus* spp. Pages 37-48 in: Proc. Biological Control of Postharvest Diseases of Fruits and Vegetables Workshop. C. L. Wilson and E. Chalutz, eds. U.S. Dep. Agric. Agric. Res. Serv. ARS-92.
12. Sanchez, E. E., Righetti, T. L., Sugar, D., and Lombard, P.B. 1992. Effects of timing of nitrogen application on nitrogen partitioning between vegetative, reproductive, and structural components of mature 'Comice' pears. J. Hortic. Sci. 67:51-58.
13. Shefelbine, P. A., and Roberts, R. G. 1990. Population dynamics of *Cryptococcus laurentii* in wounds in apple and pear fruit stored under ambient or controlled atmospheric conditions. (Abstr.) Phytopathology 80:1020.
14. Sitton, J. W., and Patterson, M. E. 1992. Effect of high-carbon dioxide and low-oxygen controlled atmospheres on postharvest decays of apples. Plant Dis. 76:992-995.
15. Spotts, R. A. 1985. Effect of preharvest pear fruit maturity on decay resistance. Plant Dis. 69:388-390.
16. Spotts, R. A., and Cervantes, L. A. 1986. Populations, pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* in packinghouses. Plant Dis. 70:106-108.
17. Sugar, D., Powers, K. A., and Hilton, R. J. 1991. Enhanced resistance to side rot in pears treated with calcium chloride during the growing season. Plant Dis. 75:212-214.
18. Sugar, D., Righetti, T. L., Sanchez, E. E., and Khemira, H. 1992. Management of nitrogen and calcium in pear trees for enhancement of fruit resistance to postharvest decay. HortTechnology 2:382-387.
19. Sugar, D., Roberts, R. G., Powers, K. A., and Basile, S. A. 1992. Integration of cultural and biological methods for control of postharvest decay in pear. (Abstr.) Phytopathology 82:1076.
20. Sugar, D., and Spotts, R. A. 1993. Dispersal of inoculum of *Phialophora malorum* in pear orchards and inoculum redistribution in pear immersion tanks. Plant Dis. 77:47-49.
21. Sugar, D., and Spotts, R. A. 1993. The importance of wounds in infection of pear fruit by *Phialophora malorum* and the role of hydrostatic pressure in spore penetration of wounds. Phytopathology 83:1083-1086.
22. Willett, M. J., Kupferman, E. A., Roberts, R. G., Spotts, R. A., Sugar, D., Apel, G., and Ewart, H. W. 1992. Integrated management of postharvest diseases and disorders of apples, pears, and cherries. Tree Fruit Postharvest J. 3:3-16.
23. Williams, M. W., Couey, H. M., Moffitt, H., and Coyier, D. L. 1978. Pear production. U.S. Dep. Agric. Handb. 526.