Annual Report - 2014

Prepared for the California Pear Board

Project Title:	Evaluation of Postharvest Treatments for Management of Gray Mold, Blue Mold, and other Decays of Stored Pears in California
Project Leader:	Dr. James E. Adaskaveg, Department of Plant Pathology, University of California, Riverside, CA 92521 (951) 827-7577
Cooperators:	Dr. H. Förster, D. Thompson, and D. Cary (UC Riverside)
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MAIN ACHIEVEMENTS IN 2014 RESEARCH

Our pear postharvest research focused on new treatments for the management of decays to provide solutions for conventionally treated and potentially also for organic fruit production. Treatments are being developed for long-term usage because they are integrated with anti-resistance strategies. The final pre-mixture formulation of fludioxonil and difenoconazole (Academy), the bio-fungicide polyoxin-D (proposed trade name is Post-Doc), and the new experimental EXP-13 that has a different mode of action from all other postharvest fungicides were evaluated. The latter two fungicides potentially could be registered with an exempt-from tolerance status in the US and may even be registered as organic fungicides. Laboratory and experimental packingline studies were conducted using inoculated Bosc and Comice pear fruit.

- The new pre-mixture Academy continued to be highly effective against gray mold and blue mold. We previously demonstrated a broad spectrum of activity of this pre-mixture that includes blue mold, gray mold, bull's eye rot, and Alternaria rot. This fungicide will be registered in 2015 as a postharvest treatment of pome fruit. Canada held up the joint registration with US-EPA to evaluate lower rates.
- A mixture of polyoxin-D with a low rate (150 ppm) of fludioxonil effectively reduced the incidence of gray mold and Alternaria rot to very low levels. The high efficacy of a polyoxin-D/fludioxonil mixture against blue mold was demonstrated previously.
- EXP-13 effectively reduced the incidence of gray mold, Alternaria rot, bull's eye rot, and Mucor decay, and blue mold on Bartlett pears. EXP-13, however, was not effective against blue mold on Bosc or Comice pears. Thus, we are currently investigating the reason for the lack of efficacy of EXP-13 against blue mold of selected pear varieties. Overall, the fungicide has a fairly wide spectrum of activity against different fungal pathogens. In mixture with a low rate (150 ppm) of Scholar, the treatment was highly effective against all five postharvest decays.
- In baseline sensitivity studies, EC_{50} values for inhibition of mycelial growth by EXP-13 were determined for 62 isolates of *P. expansum* from pome fruit. Sensitivity ranged from 0.774 to 1.545 ppm (average 1.143 ppm).
- In a commercial packinghouse study, long-term ozone storage reduced decay lesion size, fungal sporulation, and fungal surface growth when inoculum of the decay pathogens *B. cinerea* or *P. expansum* was used. Incidence of decay was the same for ozone and no ozone treatments. For the common surface molds *Alternaria* and *Cladosporium* spp., however, no benefits in reducing decay or growth and sporulation were observed.

INTRODUCTION

Gray mold, caused by *Botrytis cinerea*, and blue mold, caused by *Penicillium expansum* and several less common species of *Penicillium*, are the most important storage diseases of pears in California. Other decays that may cause significant losses include Alternaria, Phomopsis, Rhizopus and Mucor rots, as well as occasionally bull's eye rot caused by *Neofabraea* spp. Entry points for all pathogens are wounds caused by abiotic or biotic agents before or during harvest. On Bartlett pears, infections by *B. cinerea* during bloom often result in calyx end-rot. Infections by *Neofabraea* spp. mostly are initiated in the orchard during fruit development. While some postharvest decay fungi like *Rhizopus* species are suppressed at storage temperatures of 0°C (32°F), *B. cinerea, P. expansum,* as well as *Mucor, Alternaria*, and *Neofabraea* spp. will still grow, although slowly. Thus, additional chemical treatments are needed. Preharvest treatments with fungicides (e.g., Ziram, Captan, Pristine, Elevate) to manage postharvest decays have been inconsistent and generally unsatisfactory in their efficacy when fruit are sanitized and washed immediately after harvest. These treatments, however, can reduce the incidence of postharvest gray mold when field bins of fruit are not washed and placed directly into cold storage but they may increase the likely-hood of selecting for resistance of postharvest pathogens by over-usage (number of applications per season) and incomplete coverage. Fungicides are more effectively used as postharvest treatments. Postharvest fungicides including Penbotec (pyrimethanil - 2005), Scholar (fludioxonil - 2005) and Judge (fenhexamid – 2007) were developed by us and others because Captan at the registered postharvest rate of 2 lb/200,000 lb is ineffective against blue mold and resistance against TBZ (Mertect 340F) is widespread in populations of *B. cinerea* and *P. expansum.*

The risk of fungicide resistance development in the postharvest pear pathogens is high because most registered materials have a single-site mode of action and because fruit are stored for extended periods of time. Furthermore, when fruit receive more than one postharvest treatment, repeated selection allows the survivors to become the dominant pathogen population. Although five fungicides (Captan, TBZ, Scholar, Penbotec, Judge) are now registered for postharvest use on pears, only two of them (Scholar, Penbotec) are highly effective against TBZ-resistant blue mold. Difenoconazole that is very effective against blue mold is pending registration on pome fruit. This fungicide will be available in a pre-mixture with fludioxonil. The use of pre-mixtures is an effective anti-resistance strategy. Our laboratory selection studies indicated that pyrimethanil and fludioxonil have a similar high risk to develop resistance, but the risk for difenoconazole was determined to be lower, but still present. We have been optimizing usage rates and application methods for difenoconazole, and we evaluated different formulations of a pre-mixture for managing gray mold, blue mold, Alternaria rot, and bull's eye rot.

As additional postharvest treatment alternatives, we are evaluating the bio-fungicide polyoxin-D that has obtained an exempt registration status in the US and the experimental EXP-13 that also has potential for an exempt status and an organic registration because it is a natural fermentation product. The registrant of Scholar, Syngenta Crop Protection, will obtain the postharvest registration for polyoxin-D in the United States. This compound is very effective against gray mold and Alternaria rot and it would be a resistance management treatment when used in mixture with Scholar. EXP-13 has been used for many years in the food industry for preventing Penicillium molds and resistance has never occurred against this compound. This makes this compound an excellent candidate as a postharvest resistance management treatment.

Thus, in 2014, our pear postharvest research focused on new treatments for the management of major decays to provide solutions for conventionally treated and potentially also for organic fruit production. Treatments are being developed for long-term usage because they are integrated with anti-resistance strategies.

Objectives

1) Comparative evaluation of new postharvest fungicides
   - i) Evaluate the new difenoconazole-fludioxonil pre-mixture at selected rates against gray mold, blue mold, Alternaria decay, and bull's eye rot and compare to pyrimethanil.
   - ii) Evaluate polyoxin-D against gray mold, Alternaria decay, and bull's eye rot and compare to pyrimethanil and fludioxonil.
   - iii) Evaluate formulations of EXP-13 against blue mold, gray mold, Alternaria decay, Mucor decay, and bull's eye rot.
2) Optimize efficacy of polyoxin-D and EXP-13 by determining their post-infection activity at different times after inoculation and by evaluation of fruit of different maturity.
3) Determination of baseline sensitivities. Development of baseline sensitivities for fludioxonil and difenoconazole will be continued for additional isolates of *Alternaria* spp. that are collected. Baselines for polyoxin-D and EXP-13 will be developed upon the registrants' approval.
4) Efficacy of ozone treatments in commercial storage rooms – Evaluate the effectiveness for decay control of inoculated and for sanitizing contaminated fruit.

MATERIALS AND METHODS

Comparative evaluation of new postharvest fungicides. The efficacy of Academy (the final formulation of a difenoconazole-fludioxonil pre-mixture), polyoxin-D (CX10440 or Post-Doc), EXP-13 (two formulations: a 50% powder and a 5% liquid formulation), and selected mixtures was evaluated in laboratory and experimental packingline studies. Comice or Bosc pears were wound-inoculated with decay pathogens of pears: *P. expansum*, *B. cinerea, Neofabraea perennans, Mucor piriformis,* or *Alternaria alternata* using spore concentrations as indicated in the figures of the results. Fruit were incubated for selected times at 20°C and then treated. In laboratory studies, treatments were done by dipping for 15 sec or by hand-spraying. In experimental packingline studies, treatments were done using high-volume, in-line drench or -spray (T-Jet) applications that were followed by low-volume spray applications with fruit coating (Decco 231, a carnauba-based coating). After treatment, fruit of all studies were stored at 20°C, 95% RH for 6 to 14 days and then evaluated for the incidence of decay. Data were analyzed using analysis of variance and least significant difference mean separation procedures of SAS 9.4.

Determination of baseline sensitivities. A baseline sensitivity range for EXP-13 was established for 62 isolates of *P. expansum* from pome fruit. Concentrations to inhibit mycelial growth by 50% were determined on amended potato dextrose agar using the spiral gradient dilution method as described previously.

Efficacy of ozone treatments in commercial storage rooms for decay control of inoculated and for sanitizing contaminated fruit. Bosc pear fruit were inoculated into side wounds with conidia of *B. cinerea* (5×10^5 conidia/ml), *P. expansum* (1×10^6 conidia/ml), *A. alternata* (1.3×10^5 conidia/ml), or *Cladosporium* sp. (1×10^6 conidia/ml), or non-wound drop-inoculated on the blossom or stem end and were incubated for 16-18 h at 20C. Three layers of fruit for each treatment were left non-inoculated for development of natural incidence of decay. Fruit for ozone treatment were taken to the packinghouse where they were stored in an ozonated environment (60 ppb) at 1-2C for 7 weeks. Control fruit were incubated at 3-4C during without ozone for the same time period. Wound inoculation sites were evaluated for incidence and severity (lesion diameter) of decay and for growth and sporulation at the inoculation sites using a scale from 0 to 2 with 0 = no growth, 1 = mycelial growth present, and 2 = mycelial growth present and fungal sporulation. Fruit for development of natural incidence of decay were then incubated for an additional 10 days at 20C.

RESULTS AND DISCUSSION OF 2014 RESEARCH

Comparative evaluation of new postharvest fungicides. In laboratory studies, EXP-13 and polyoxin-D (formulation CX-10440 or Post-Doc) mixed with Scholar were very effective in reducing the incidence of gray mold and Alternaria rot when fruit were treated 16 h after inoculation (Fig. 1). Addition of the detergent Tween 80 to EXP-13 caused a trend for reduced activity against gray mold (in studies on postharvest decays of other crops, the addition of Tween 80 had an inconsistent effect on efficacy). EXP-13 (similar to polyoxin-D, as previously reported) was not effective against blue mold on cvs. Bosc or Comice fruit even when they were treated only 6 h after inoculation (Figs. 1,2). When EXP-13 was mixed with Scholar at a low rate (150 ppm), the incidence of all three decays was significantly reduced from those of the controls (Fig. 1).

In two experimental packingline studies, EXP-13 was highly effective (Fig. 3) or effective (Fig. 4) in reducing gray mold and again was not effective against blue mold on Comice or Bosc pear (Figs. 3,4), respectively. The compound also significantly reduced the incidence of bull's eye rot and Mucor decay (Fig. 4), but showed little effectiveness against Alternaria rot as in the laboratory study. Thus, EXP-13 has a fairly wide spectrum of activity against different fungal pathogens. In mixture with a low rate of Scholar, the treatment was highly effective against all five postharvest decays. The new fludioxonil-difenoconazole pre-mixture Academy will be registered in 2015. Canada held up the joint registration with US-EPA to evaluate lower rates (e.g., 8 oz). In our trials, the fungicide was tested only at 16 fl oz/100 gal. Academy was highly effective against gray mold and blue mold (Fig. 3). In last year's studies, Academy demonstrated high efficacy also against bull's eye rot and Alternaria rot. Difenoconazole will only be sold in a premixture with fludioxonil and will not be sold by itself.

For Academy, the in-line drench application was significantly more effective in reducing the incidence of blue mold (Fig. 3), similar as we demonstrated previously in numerous other packingline studies. Interestingly for EXP-13, however, there was no significant difference in using T-jet and in-line drench applications for all pathogens and in both studies (Figs. 3,4). Apparently, threshold concentrations of EXP-13 reduce postharvest decays, and optimized application methods or using increased rates do not improve efficacy.

In other studies, we also tested EXP-13 using apple fruit and interestingly, for all five decays, efficacy was higher on Granny Smith apple fruit than on Bosc or Comice pears. Blue mold of Granny Smith apple was reduced by up to 90% as compared to control fruit and EXP-13 was also effective against this decay on Fuji apple. This difference in efficacy led to investigations as of why the efficacy of EXP-13 was higher on apples than on pears. Several approaches were pursued. First, in vitro sensitivity of *P. expansum* against EXP-13 was compared using apple- or pear-based agar media and EC_{50} values for mycelial growth were approximately 10 lower using apple or pear medium than using standard potato dextrose agar. Therefore, these results could not explain the differences in efficacy on pear and apple. Subsequently, we compared conidial germination and germ tube growth on apple and pear fruit and in apple or pear juice. Germ tubes developed faster on pear than on apple tissue and after 24 h germ tubes were approximately 30% longer on the pear substrate. Because treatments to fruit were applied sometimes only 6 h after inoculation when spores of *P. expansum* are not even starting to germinate, this difference in germ tube grow cannot be responsible for the different efficacy of EXP-13 on pear and apple. Therefore, we cannot explain these differences. Currently there is an indication, however, that pH may have a role on the fungicides performance. The activity of EXP-13 is pH dependent and is higher in the more acidic range. Fruit tissue of apple has a lower pH than that of pear. Furthermore, we have also obtained very good efficacy with this compound on citrus fruit for control of Penicillium decay. We are currently evaluating EXP-13 solutions with different pH on inoculated pear fruit and results are pending.

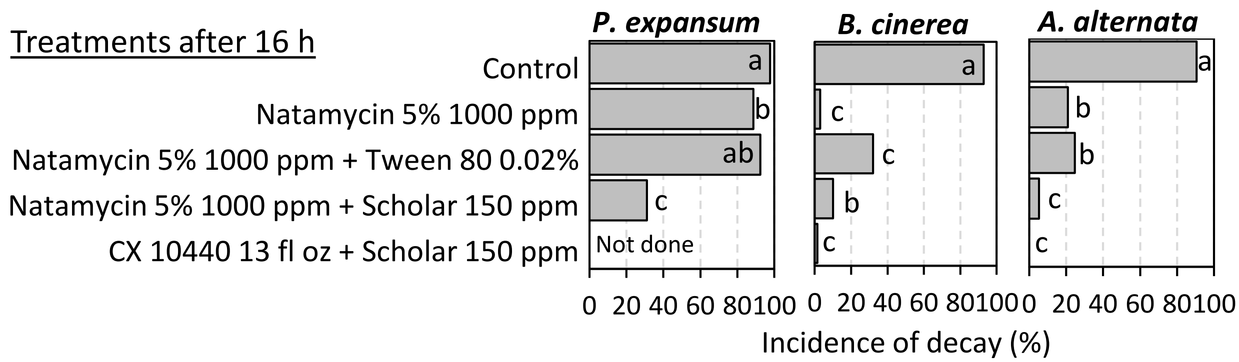
Thus, polyoxin-D and EXP-13 will have to be continued to be evaluated. Syngenta Crop Protection is interested in being the registrant for both compounds in the US. Additional research is important because both will be potentially exempt from tolerance and the registrants are requesting use for organic fruit production since both are fermentation products. They also could be used in mixtures to prevent resistance of gray mold to fludioxonil. Fludioxonil is currently the only highly effective gray mold material used commercially where no resistance has been found. Thus, its activity needs to be protected.

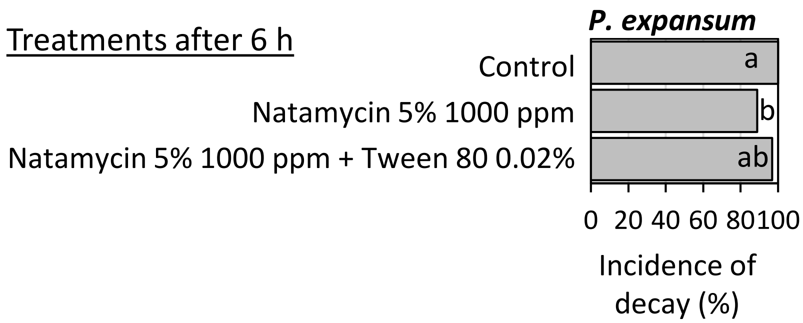
Determination of baseline sensitivities. EC_{50} values for inhibition of mycelial growth by EXP-13 were determined for 62 isolates of *P. expansum* from pome fruit (Fig. 6). There was a narrow range of sensitivity from 0.774 to 1.545 ppm (average 1.143 ppm). Low variation in a diverse population evaluated is a favorable anti-resistance characteristic of a fungicide. Sensitivity ranges for other decay pathogens will need to be done in the future.

Efficacy of ozone treatments in commercial storage rooms for decay control of inoculated and for sanitizing contaminated fruit. Wound-inoculated fruit stored for 7 weeks in an ozone atmosphere in a commercial packinghouse developed a similar incidence of gray mold and blue mold as control fruit stored at a slightly higher temperature (3-4°C as compared to 1-2°C) in a non-ozonated environment (Fig. 7A,B). Lesion diameter and sporulation rating, however, were significantly smaller for the ozone-treated fruit and additionally, fungal growth and sporulation on the drop-inoculated blossom end and on the cut stem surface were also significantly reduced on the treated fruit. Incidence of Alternaria decay of wound-inoculated fruit was low (9.1% in the control, 24.3% in the ozone-treatment) and was significantly higher on the ozone-treated fruit (Fig. 7C). No decay developed after wound-inoculation with *Cladosporium* sp. in ozone and no ozone treatments. In contrast to gray mold and blue mold, there were no significant differences in fungal growth and sporulation on the inoculation sites for both *Alternaria* and *Cladosporium*-inoculated fruit (Fig. 7C,D). After cold-temperature, ozone or ambient-atmosphere incubation, *Alternaria*-inoculated fruit were incubated for an additional 10 days at 20C at ambient atmosphere and incidence of decay increased to 90.5% in fruit of the control (no ozone) treatment and 100% in the ozone-treated fruit and lesion diameter was significantly higher on the ozone treated fruit (9.3 mm vs. 14.7 mm). No natural incidence of decay developed on any of the fruit for 7 weeks but after an additional 10-day ambient incubation, 57.7% of the ozone-treated fruit developed Penicillium decays, whereas no decay was present on the control fruit.

These data indicate that long-term ozone storage can have some benefits in reducing the size of decay lesions, fungal sporulation, and growth of surface mold when *B. cinerea* or *P. expansum* inoculum is present. For the common surface molds *Alternaria* and *Cladosporium* spp., however, no benefits were observed. Subsequent incubation of ozone-stored fruit in non-ozone and warm environments (e.g., 20C) resulted in higher decay. This may indicate that the fruit may have senesced in the ozone environment with a concurrent increase in host susceptibility.

Fig. 1. Evaluation of new postharvest treatments for management of postharvest decays of Bosc pear in laboratory studies

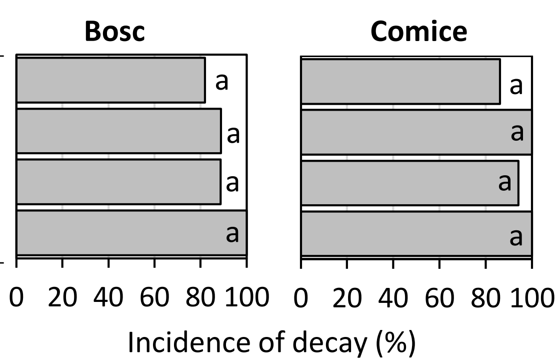




Fruit were wound-inoculated with conidia of *B. cinerea* (1×10^5/ml), *P. expansum* (2×10^5/ml), or *Alternaria alternata* (1×10^5/ml) and were incubated for 6 or 16 h at 20C. Treatments with aqueous fungicide solutions were done by hand-spraying. Fruit were then incubated at 20C for 5 to 7 days.

Fig. 2. Evaluation of post-inoculation timings of dip applications with EXP-13 for management of blue mold of Bosc and Comice pears in laboratory studies

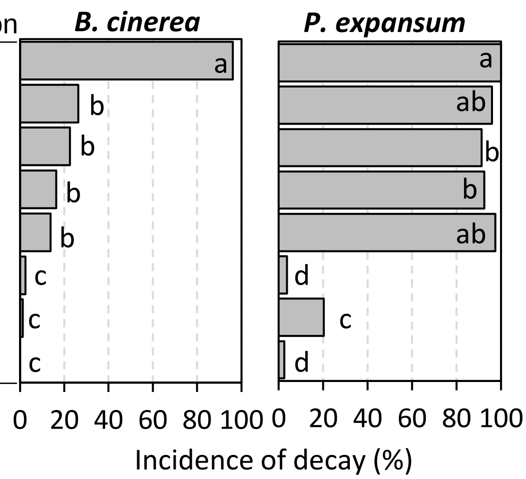
Treatment	Rate	Timing (after inoculation)
Control (water)	---	4 h
EXP-13 SC	1000 ppm	4 h
EXP-13 SC	1000 ppm	8 h
EXP-13 SC	1000 ppm	12 h



Fruit were wound-inoculated with conidia of *P. expansum* (1×10^5 conidia/ml) and were incubated for selected times at 20C. Treatments were done by aqueous 15-sec dips. Fruit were then incubated at 20C for 5 to 7 days.

Fig. 3. Evaluation of new postharvest treatments for management of gray mold and blue mold of Comice pear in an experimental packingline study

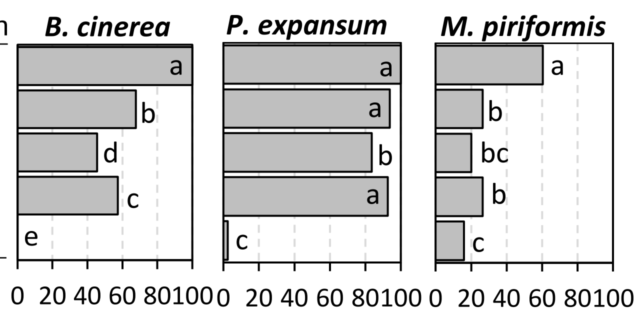
Treatment	Rate	Application
Control (water)	---	Drench
EXP-13 WP	1000 ppm	T-Jet
EXP-13 SC	1000 ppm	T-Jet
EXP-13 SC	1000 ppm	Drench
EXP-13 WP	1000 ppm	Drench
EXP-13 WP + Scholar	1000+150 ppm	Drench
Academy	16 fl oz	T-Jet
Academy	16 fl oz	Drench



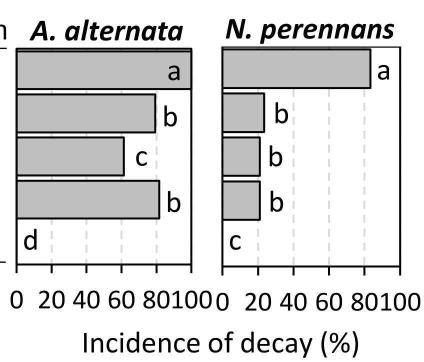
Fruit were wound-inoculated with conidia of *B. cinerea* (5 x 10^4 conidia/ml) or *P. expansum* (5 x 10^5 conidia/ml) and were incubated for 16-18 h at 20C. Treatments with aqueous fungicide solutions were done by in-line drench or by T-jet applications and were followed by a CDA application with carnauba fruit coating (Decco 231). Fruit were then incubated at 20C for 5 to 7 days. 16 fl oz Academy = 180 ppm fludioxonil + 300 ppm difenoconazole).

Fig. 4. Evaluation of EXP-13 for management of postharvest decays of Bosc pear in experimental packingline studies

Treatment	Rate	Application
Control (water)	---	Drench
EXP-13 WP	1000 ppm	T-Jet
EXP-13 SC	1000 ppm	T-Jet
EXP-13 WP	1000 ppm	Drench
EXP-13 WP + Scholar	1000+150 ppm	Drench

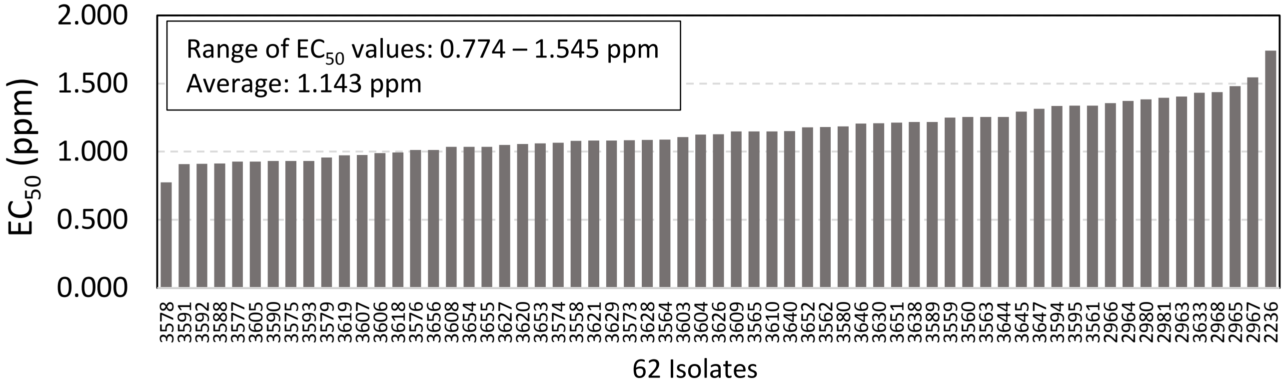


Treatment	Rate	Application
Control (water)	---	Drench
EXP-13 WP	1000 ppm	T-Jet
EXP-13 SC	1000 ppm	T-Jet
EXP-13 WP	1000 ppm	Drench
EXP-13 WP + Scholar	1000+150 ppm	Drench



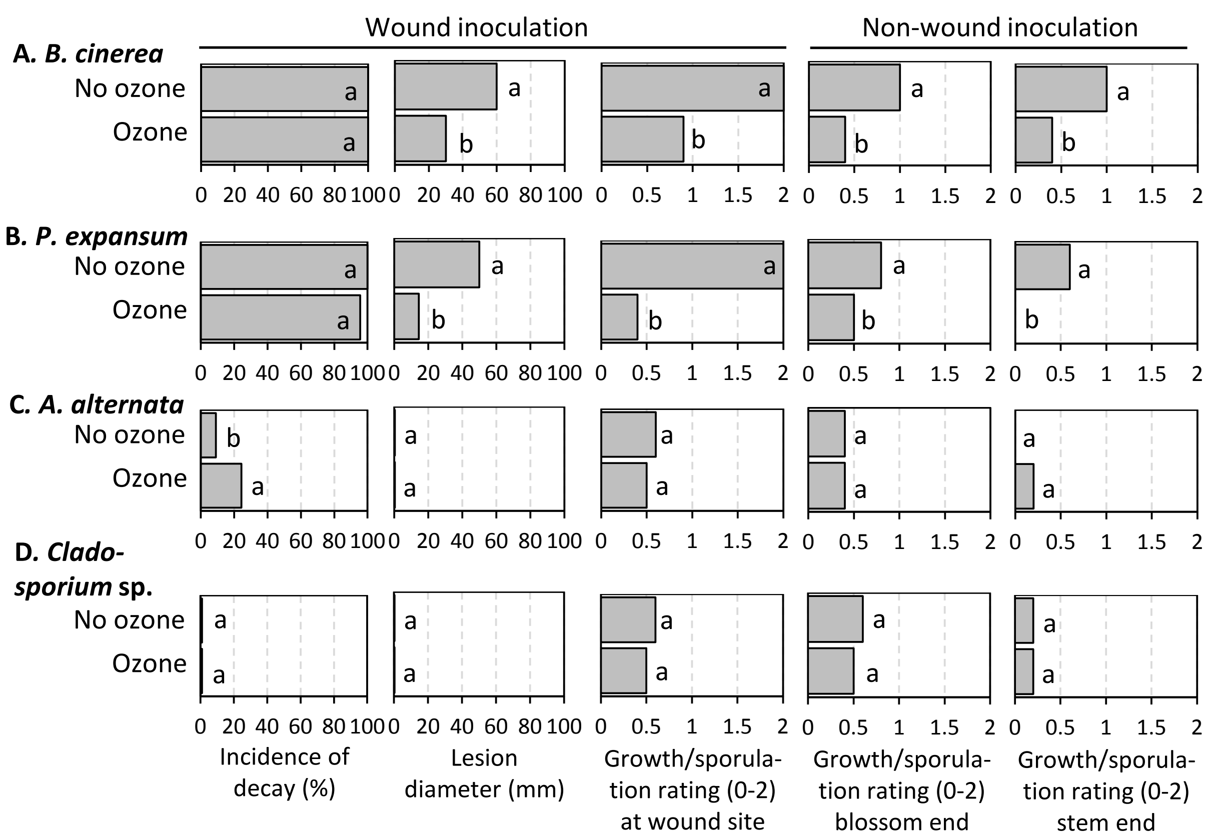
Fruit were wound-inoculated with conidia of *B. cinerea* (5 x 10^4 conidia/ml), *P. expansum* (5 x 10^5 conidia/ml), *Mucor piriformis* (5 x 10^5 spores/ml), *Alternaria alternata* (1 x 10^5 conidia/ml), or *Neofabraea perennans* (1 x 10^6 conidia/ml), and were incubated for 16-18 h at 20C. Treatments with aqueous fungicide solutions were done by in-line drench or by T-jet applications and were followed by a CDA application with carnauba fruit coating (Decco 231). Fruit were then incubated at 20C for 5 to 7 days.

Fig. 6. Baseline sensitivity range for mycelial growth of 62 isolates of *P. expansum* against EXP-13



Concentrations to inhibit mycelial growth by 50% were determined using the spiral gradient dilution method.

Fig. 7. Evaluation of ozone storage treatments for management of postharvest fungal growth and decays of Bosc pear in a commercial packinghouse study



Fruit were inoculated into side wounds with conidia of *B. cinerea* (5 x 10^5 conidia/ml), *P. expansum* (1 x 10^6 conidia/ml), *Alternaria alternata* (1.3 x 10^5 conidia/ml), or *Cladosporium* sp. (1 x 10^6 conidia/ml), or non-wound drop-inoculated on the blossom or stem end and were incubated for 16-18 h at 20C. Control fruit were incubated at 6.5C. Fruit for ozone treatment were taken to the packinghouse where they were stored in an ozonated environment at 1-2C. Fruit were evaluated after 7 weeks. No natural incidence of decay was present on any of the fruit.