Annual Report - 2013

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
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MAIN ACHIEVEMENTS IN 2013 RESEARCH

- 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. The final pre-mixture formulation of fludioxonil and difenoconazole (Academy), the new multi-pack formulation of fludioxonil and TBZ (Scholar Max MP), the bio-fungicide polyoxin-D, and a new active ingredient for postharvest decay control (i.e., N-1) were evaluated.
- Experimental packingline studies on three pear cultivars (Bartlett, Bosc, Comice) again demonstrated that re-circulating high-volume drench applications are the most effective method to provide excellent coverage and decay control.
- Applications with Academy, Scholar Max MP, Scholar, and Penbotec were all highly effective in reducing blue mold (caused by TBZ-sensitive and -resistant strains of *P. expansum*) and gray mold. Academy and Scholar Max MP also reduced the incidence of bull's eye rot to low levels.
- Academy and Scholar were highly effective against Alternaria rot. This further broadens the spectrum of activity of the fludioxonil-difenoconazole pre-mixture Academy with blue mold, gray mold, bull's eye rot, and Alternaria rot.
- Polyoxin-D that has an exempt registration status was less effective against gray mold in this year's studies as compared to last year. It is suggested that fruit cultivar and maturity used may be critical for the optimum performance of this compound and these parameters need to be further evaluated.
- N-1 showed moderate efficacy against gray mold and good efficacy against Mucor rot. The compound was more effective when treatments were applied after shorter post-inoculation incubation periods. As with polyoxin-D, further studies are needed to optimize the performance of this compound. This is important because both polyoxin-D and N-1 potentially could be used for organic fruit production and they also could be used in mixtures to prevent resistance of gray mold to fludioxonil.

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 and they may increase the likely-hood of selecting for resistance of postharvest pathogens 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.* These treatments are just recently being utilized in packinghouses because many countries had to establish maximum residue limits (MRLs) that allow import of California grown fruit that were postharvest treated with these fungicides.

The risk of resistance development in the postharvest pear pathogens to fungicides 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. Our laboratory selection studies indicated that the latter two fungicides have a similar high risk to develop resistance. For difenoconazole, no resistant isolates could be selected and the resistance potential was concluded to be lower, but still present. Anti-resistance strategies include the use of fungicide rotations and mixtures. For this, we are identifying additional potential postharvest fungicides, and we continued our evaluation of the sterol biosynthesis inhibitor difenoconazole. We have been working in close collaboration with the registrant of Scholar and difenoconazole, Syngenta Crop Protection, who is very supportive of these studies. One goal is to ultimately provide a pre-mixture of these fungicides that is both highly efficacious and cost-effective. For this, we are optimizing usage rates, application methods, and we are evaluating different formulations of a pre-mixture for managing gray mold, blue mold, Alternaria rot, and bull's eye rot. Although this latter decay is only of sporadic importance in California (but very important in the Pacific Northwest), management strategies need to be known in the event of a disease outbreak.

As an additional alternative, we are evaluating the bio-fungicide polyoxin-D that has obtained an exempt registration status in the US as a potential postharvest treatment for organic production. We obtained excellent gray mold reduction in a previous study using this compound, and in 2013 we continued its evaluation. Furthermore, another compound (N-1) was evaluated as a postharvest treatment on pome fruit and other crops. N-1 is known for its activity against *Penicillium* species growing on dairy products, where it has been used as a food additive for many years. The compound has the potential to obtain an exempt status and an organic registration because it is a natural fermentation product. Furthermore, over all the years in use, resistance in *Penicillium* species against N-1 has not occurred. N-1 was never evaluated on pome fruit and thus, we conducted studies on its use as a postharvest treatment.

These latter two alternative treatments could also be used as components of anti-resistance management for currently registered fungicides. Thus, for fludioxonil (Scholar), difenoconazole has been developed to prevent resistance in *Penicillium* populations. Polyoxin-D or N-1 could take this role for *B. cinerea*, because difenoconazole has little activity against gray mold. Thus, in 2013, 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 difenoconazole, fludioxonil, and difenoconazole-fludioxonil pre-mixtures at selected rates against gray mold, blue mold, Alternaria decay, and bull's eye rot and compare to pyrimethanil.
    ii) Compare application methods for the difenoconazole-fludioxonil pre-mixture: in-line drench, T-Jet, and CDA applications on an experimental packingline.
    ii) Evaluate polyoxin-D against gray mold, Alternaria decay, and bull's eye rot and compare to pyrimethanil and fludioxonil.
    iii) Evaluate N-1 at selected rates and determine the spectrum of activity for the different postharvest pear decays. Determine post-infection activity at different times after inoculation.
2) Evaluate treatment effects on fungicide residues on pear fruit – determine the effect of temperature differences between treatment solution and fruit on uptake of fludioxonil and difenoconazole of different pear cultivars.
3) Determination of baseline sensitivities. Baseline sensitivities for fludioxonil and difenoconazole will be continued to be developed for additional isolates of *Alternaria* spp. that are collected.

MATERIALS AND METHODS

Efficacy of postharvest treatments and application methods using single fungicides and mixtures. The efficacy of Academy (the final formulation of a difenoconazole-fludioxonil pre-mixture), Scholar Max MP (a new multi-pack formulation of fludioxonil and TBZ) was evaluated in comparison with Scholar 1230SC and Penbotec. Applications were done using high-volume in-line drench and low-volume CDA spray applications on an experimental packingline using the suggested commercial rates. Bartlett, Comice, or Bosc pears were wound-inoculated with TBZ-resistant isolates of *P. expansum* ($5x10^5$ conidia/ml), or with *B. cinerea* (10^5 conidia/ml), *Neofabraea perennans* (10^6 conidia/ml), or *Alternaria alternata* (105 conidia/ml), incubated for 15-17 h at 20°C, and then treated. Fungicides were applied on an experimental packingline at the Kearney Agricultural Center as aqueous solutions using in-line drench applications that were followed by low-volume spray applications with fruit coating (Decco 231, a carnauba-based coating) or by low-volume CDA spray application at a rate of 25 gal/200,000 lb fruit. For N-1, two formulations (a 50% powder and a 5% liquid formulation) were used and applications were done using either method or a combination of the two.

A timing study was conducted with polyoxin-D and N-1 on apple fruit. Fruit were inoculated and incubated for selected times (4, 6, 9, or 12 h) at 20C. Treatments with aqueous fungicide solutions were done using a hand-sprayer. 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 9.1.

RESULTS AND DISCUSSION OF 2013 RESEARCH

Evaluation of postharvest treatments using single-fungicides, mixtures, and pre-mixtures. Experimental packingline studies on three pear cultivars (Bartlett, Bosc, Comice) were conduced to evaluate new pre-mixture treatments in comparison with single-fungicides, as well as polyoxin-D and a new active ingredient for postharvest decay management, i.e., N-1. Decays studied included blue mold, gray mold, bull's eye rot, and Alternaria rot. The latter decay can be quite serious on injured pome fruit, but was never before included in out postharvest studies.

For the evaluation of the final pre-mixture formulation of fludioxonil-difenoconazole (i.e., Academy) and of a new fludioxonil-TBZ multi-pack formulation (i.e., Scholar Max MP) in comparison with fludioxonil (Scholar) alone, the efficacy was compared using re-circulating in-line drench and low-volume, CDA spray applications (Figs. 1-3). In most cases, the efficacy of each treatment was much increased when applied as a drench application. During the application on a roller bed, the pear fruit did not rotate well on the roller bars, and thus, fungicide coverage of the inoculation site was poor using a low-volume spray application. Under commercial conditions, certain devices are used to improve fruit rotation during fungicide application, and thus, efficacy of low-volume applications is likely higher. Still, re-circulating high-volume drench applications are the most effective method to provide excellent coverage and decay control.

In-line drench applications with Academy, Scholar Max MP, Scholar, and Penbotec were all highly effective in reducing blue mold (caused by TBZ-sensitive and -resistant strains of *P. expansum*) and gray mold of Bartlett and Bosc pear (Figs. 1 and 2). Decay incidence was reduced from over 80% in the control to less than 6% in most cases. As indicated previously, Scholar is not effective against bull's eye rot. However, the fludioxonil-difenoconazole mixture Academy and the fludioxonil-TBZ mixture Scholar Max reduced the incidence of decay to low levels on Bosc pear inoculated with *N. perennans* (Fig. 2) and Comice pear inoculated with *N. malicorticis* (Fig. 3). Penbotec was only highly effective against this decay on Bosc pear. Resistance against pyrimethanil has developed in some populations of the three decay fungi at some locations and thus, this fungicide has to be rotated with different modes of action. Although difenoconazole is not effective against gray mold, and generally did not provide an additive effect in blue mold control when used in mixtures with Scholar as compared to using Scholar alone, registration of the pre-mixture will be an important tool to decrease the risk of fungicide resistance to develop in populations of *Penicillium* spp.

For control of Alternaria rot where all non-fungicide-treated fruit became infected, Academy completely prevented the development of decay (Fig. 3). This treatment also resulted in numerically the lowest incidence of decay in a similar study on apple. Scholar by itself was also very effective, but the mixture with TBZ was less effective. This could possibly indicate a negative interaction between the two fungicides, but in the study with apple, this interaction was not observed. Difenoconazole and fludioxonil were tested last year for their in vitro activity against *Alternaria* sp. and the low EC_{50} values obtained (0.01 to 0.04 ppm for difenoconazole, 0.011 to 0.025 ppm for fludioxonil) support their high effectiveness against this decay. The Penbotec treatment resulted in some reduction of decay on Comice pear, but is was very effective in the study on apple. Thus, for control of Alternaria rot of pear, Scholar and Academy were most effective and this further broadens the spectrum of activity of the fludioxonil-difenoconazole pre-mixture with blue mold, gray mold, bull's eye rot, and Alternaria rot. Studies on Alternaria rot will need to be repeated next year.

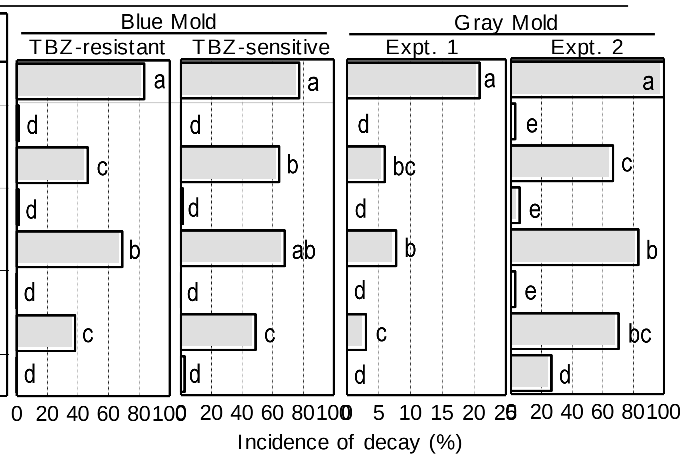
In a postharvest packingline study with CX-10440 (polyoxin-D), this treatment was not effective against gray mold when used by itself, and Alternaria rot was only reduced by approximately 30% (Fig. 4). In contrast, Alternaria rot on apple fruit that were treated together with the pears was reduced by 90%, and polyoxin-D also significantly reduced the incidence of gray mold as compared to the control (with a 50% reduction). This indicates that the type of fruit or its maturity are critical for the effectiveness of this treatment (see below).

N-1 is a new active ingredient for postharvest use that we evaluated for the first time in 2013. In the first study on Bartlett pear, it was highly effective against blue mold and gray mold (Fig. 5), however, in two subsequent studies on Bosc and Comice pear, good efficacy was only obtained for gray mold (Figs. 6 and 7). In combination with a low rate of Scholar (8 fl oz, = approx. 150 ppm), however, both decays were completely prevented. Interestingly, N-1 was also significantly reduced the incidence of Mucor decay caused by *M. piriformis* (Fig. 7). Thus, as with polyoxin-D, the efficacy of this compound may be highly dependent on fruit maturity and the best application strategies still need to be defined.

In a timing study where treatments with polyoxin-D or N-1 were applied to apple fruit selected times after inoculation, efficacy was shown to be highly dependent on the timing. Thus, for N-1, treatments applied 4 or 6 h after inoculation were significantly more effective than when applied after 9 or 12 h (Fig. 8). A trend for better efficacy in the 4-h timing was also observed for polyoxin-D. Considering that highly susceptible, senescent fruit were used in this latter study, higher efficacy is expected when treating fruit immediately after harvest. Thus, both compounds will have to be continued to be evaluated. This is important because both potentially could be used for organic fruit production. 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.

Fig. 1. Evaluation of postharvest applications with new fungicide pre-mixtures for management of blue and gray mold decay of Bartlett pears in experimental packingline studies

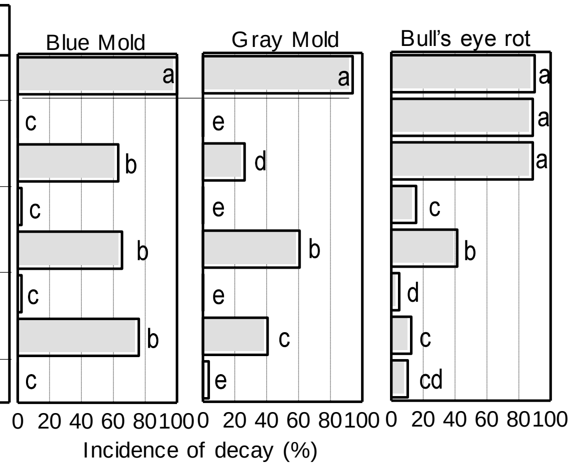
Treatment	A.i.	Method and rate
Control (water)	----	---
Scholar 230SC	fludioxonil	Drench 10 fl oz
Scholar 230SC	fludioxonil	CDA 16 fl oz
Academy 384SC	flud. + difenoc.	Drench 16 fl oz
Academy 384SC	flud. + difenoc.	CDA 16 fl oz
Scholar Max MP	flud. + TBZ	Drench 10 + 16 fl oz
Scholar Max MP	flud. + TBZ	CDA 16 + 16 fl oz
Penbotec	pyrimethanil	Drench 500 ppm



Fruit were inoculated with conidia of TBZ-sensitive or resistant isolates of *Penicillium expansum* (500,000 conidia/ml) or *B. cinerea* (100,000 conidia/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnauba fruit coating (Decco 231). CDA applications were done using 25 gal/200,000 lb fruit and treatments were done in carnauba fruit coating. Rates for CDA applications are for 200,000 lb fruit. For Scholar Max MP rates are given separately for the two components, whereas for the pre-mixture Academy the rate is given as a total of the two components. 10 fl oz Scholar = 180 ppm, 16 fl oz = 480 ppm, 16 fl oz Academy = 480 ppm = 10 fl oz Scholar + 10.7 fl oz A8574D. Fruit were then incubated at 20 C for 6 days.

Fig. 2. Evaluation of postharvestapplications with new fungicide pre-mixtures for management of blue and gray mold decay of Bosc pears in experimental packingline studies

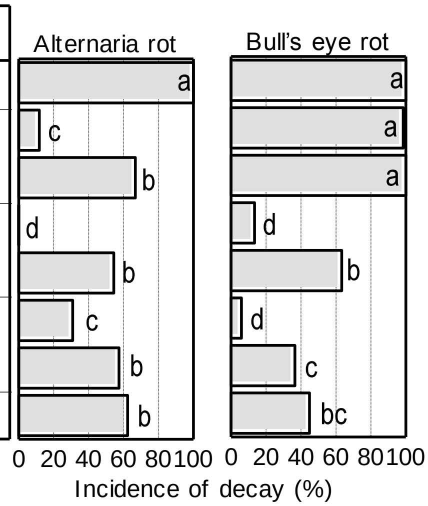
Treatment	A.i.	Method and rate
Control (water)	----	---
Scholar 230SC	fludioxonil	Drench 10 fl oz
Scholar 230SC	fludioxonil	CDA 16 fl oz
Academy 384SC	flud. + difenoc.	Drench 16 fl oz
Academy 384SC	flud. + difenoc.	CDA 16 fl oz
Scholar Max MP	flud. + TBZ	Drench 10 + 16 fl oz
Scholar Max MP	flud. + TBZ	CDA 16 + 16 fl oz
Penbotec	pyrimethanil	Drench 500 ppm



Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* (500,000 conidia/ml) or with *B. cinerea* (100,000 conidia/ml) or *N. perennans* (1,000,000 conidia/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnau fruit coating (Decco 231). CDA applications were done using 25 gal/200,000 lb fruit and treatments were done in carnauba fruit coating. Rates for CDA applications are for 200,000 lb fruit. For Scholar Max MP rates are given separately for the two components, whereas for the pre-mixture Academy the rate is given as a total of the two components. 10 fl oz Scholar = 180 ppm, 16 fl oz = 480 ppm, 16 fl oz Academy = 480 ppm = 10 fl oz Scholar + 10.7 fl oz A8574D. Fruit were then incubated at 20 C for 6 days.

Fig. 3. Evaluation of postharvestapplications with new fungicide pre-mixtures for management of Alternaria and bull's eye rot of Comice pears in experimental packingline studies

Treatment	A.i.	Method and rate
Control (water)	----	---
Scholar 230SC	fludioxonil	Drench 10 fl oz
Scholar 230SC	fludioxonil	CDA 16 fl oz
Academy 384SC	flud. + difenoc.	Drench 16 fl oz
Academy 384SC	flud. + difenoc.	CDA 16 fl oz
Scholar Max MP	flud. + TBZ	Drench 10 + 16 fl oz
Scholar Max MP	flud. + TBZ	CDA 16 + 16 fl oz
Penbotec	pyrimethanil	Drench 500 ppm

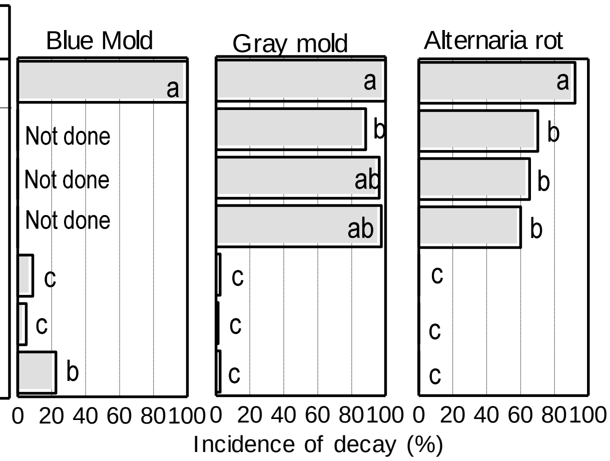


Fruit were inoculated with conidia of *Alternaria alternata* or *Neofabraea malicorticis* (100,000 conidia/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnauba fruit coating (Decco 231). CDA applications were done using 25 gal/200,000 lb fruit and treatments were done in carnauba fruit coating. Rates for CDA applications are for 200,000 lb fruit. For Scholar Max MP rates are given separately for the two components, whereas for the pre-mixture Academy the rate is given as a total of the two components. 10 fl oz Scholar = 180 ppm, 16 fl oz = 480 ppm, 16 fl oz Academy = 480 ppm = 10 fl oz Scholar + 10.7 fl oz A8574D. Fruit were then incubated at 20 C for 6 days.

Fig. 4. Evaluation of polyoxin-D (CX-10440) as a potential new postharvest treatment for management of blue mold, gray mold, and Alternaria rot of Bosc pears in experimental packingline studies

In-line drench applications

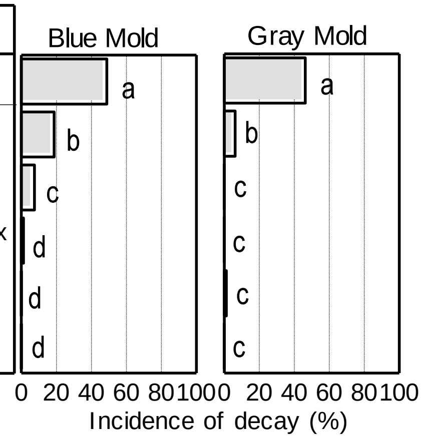
Polyoxin D	Scholar SC	Difenoconazole
Control (water)	---	---
13.5 fl oz	---	---
6.75 fl oz	---	---
3.4 fl oz	---	---
3.4 fl oz	10 fl oz	---
6.75 fl oz	10 fl oz	---
3.4 fl oz	10 fl oz	10.7 fl oz



Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* (500,000 conidia/ml), *B. cinerea* (100,000 conidia/ml) or *Alternaria alternata* (100,000 conidia/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnau fruit coating (Decco 230). For difenoconazole, the A8574D formulation was used. 10 fl oz Scholar = 180 ppm, 10.7 fl oz A8574D = 300 ppm. Fruit were then incubated at 20 C for 6 days.

Fig. 5. Evaluation of two formulations of N-1 as a potential new postharvest treatment for management of blue and gray mold decay of Bartlett pears in experimental packingline studies

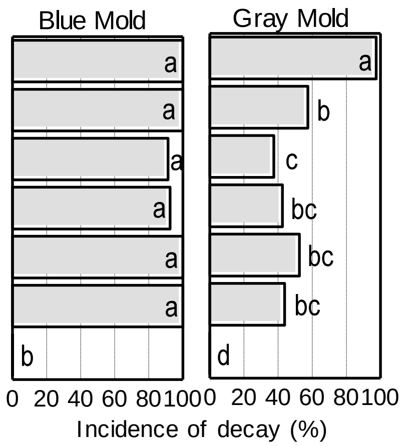
Drench application	CDA application
Control (water)	Wax only
N-1 50% form. 1000 ppm	No Wax
N-1 50% form. 1000 ppm	Wax only
N-1 50% form. 1000 ppm	N-1 5% form. 250 ppm in wax
N-1 50% form. 2000 ppm	Wax only
Scholar SC 276 ppm	Wax only



Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* (500,000 conidia/ml) or with *B. cinerea* (100,000 conidia/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnauba frui coating (Decco 231). Fruit were then incubated at 20 C for 6 days.

Fig. 6. Evaluation of two formulations of N-1 as a potential new postharvest treatment for management of blue and gray mold decay of Bosc pears in experimental packingline studies

Drench application	CDA application
Control (water)	Wax only
N-1 50% form. 1000 ppm	Wax only
N-1 5% form. 1000 ppm	Wax only
N-1 5% form. 500 ppm	N-1 5% form. 500 ppm in wax
N-1 50% form. 500 ppm	N-1 5% form. 500 ppm in wax
---	N-1 5% form. 1000 ppm in wax
N-1 50% form. 500 ppm + 8 fl oz Scholar SC	Wax only

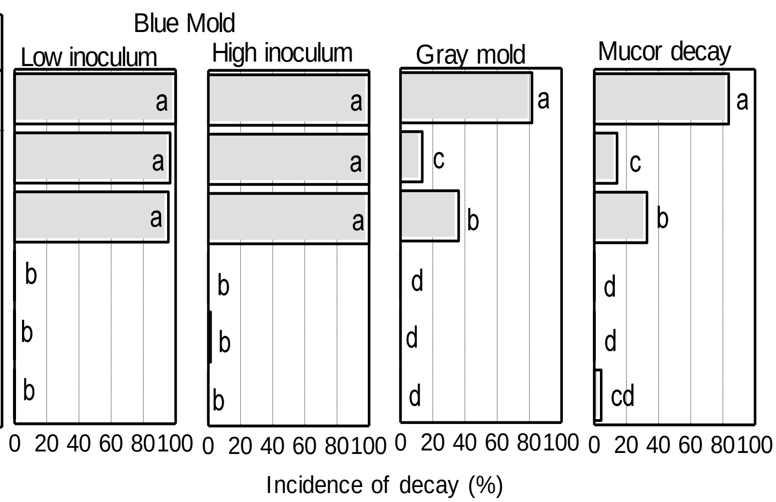


Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* (500,000 conidia/ml) or with *B. cinerea* (100,000 conidia/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnauba fruit coating (Decco 231). Fruit were then incubated at 20 C for 6 days.

Fig. 7. Evaluation of two formulations of N-1 as a potential new postharvest treatment for management of blue mold, gray mold, and Mucor decay of Comice pears in experimental packingline studies

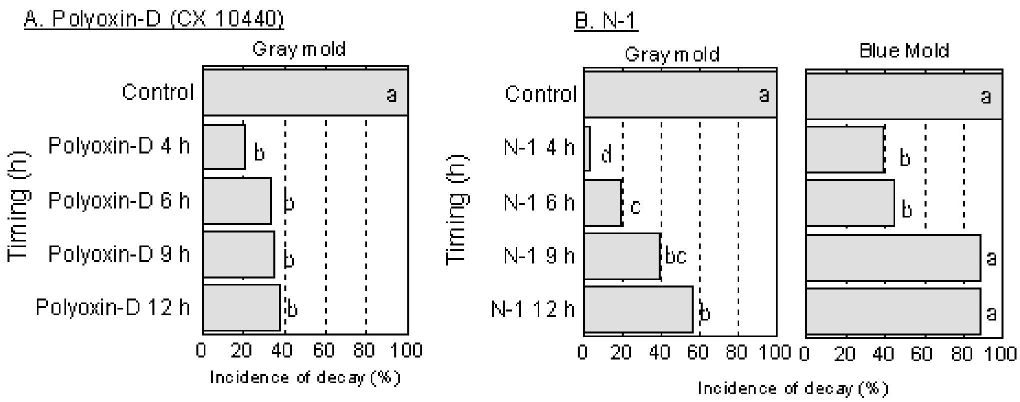
In-line drench applications

N-1	Scholar SC
Control (water)	---
50% form. 1000 ppm	---
50% form. 500 ppm	---
50% form. 500 ppm	8 fl oz
5% form. 2000 ppm	8 fl oz
---	8 fl oz



Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* (100,0000 or 500,000 conidia/ml), *B. cinerea* (50,000 conidia/ml) or *M. piriformis* (100,000 spores/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnauba fruit coating (Decco 231). Fruit were then incubated at 20 C for 6 days.

Fig. 8. Effect of application timing after inoculation on the efficacy of postharvest treatments with polyoxin-D and N-1 for management of blue mold and gray mold in laboratory studies



Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* or with *B. cinerea* (100,000 conidia/ml each) and were incubated for selected times at 20C. Treatments with aqueous fungicide solutions were done using a hand-sprayer. Fruit were then incubated at 20 C for 6 days.