Annual Report - 2006

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 2006 RESEARCH

1. Laboratory, experimental packingline, and commercial packingline studies were conducted on the management of postharvest decays of pears using new fungicides and sanitation treatments.
2. In experimental packingline studies with Bartlett and Bosc pears that were inoculated with TBZ-resistant pathogens, Scholar (using the new SC formulation at rates between 150 and 300 ppm) and Penbotec (at 500 ppm) were highly effective against blue mold caused by *P. expansum* and gray mold caused by *B. cinerea*. Low-volume spray applications were generally less effective than in-line drench or dip applications.
3. In delayed-treatment studies where fruit were inoculated 7 days before treatment and stored at ca. 2 C, Scholar and Penbotec also were very effective in reducing the incidence of decay. Treatments conducted 21 days after inoculation were done to evaluate sporulation control of the fungicides. These studies indicated that both fungicides reduced the amount of sporulation on decaying fruit. Scholar, however, was significantly more effective than Penbotec.
4. Mixtures of Scholar with Mertect or Captan were similarly effective as Scholar alone. Mertect alone reduced decay incidence caused by the TBZ-resistant pathogens by ca. 50% for gray mold and up to 20% for blue mold. Captan that was included in the fungicide mixtures as a potential companion fungicide in re-circulation application systems to inhibit spore germination was more effective against gray mold than against blue mold. Still, when used by itself, reduction in decay incidence was only up to ca. 60%.
5. In additional experimental packingline studies, chlorine was added to the fungicide solutions, again, as a potential sanitation treatment. The efficacy of both Scholar and Penbotec was not affected when chlorine was added to the fungicide solutions up to 16 h before use.
6. Acidified hydrogen peroxide (used as the product Perasan) and Captan were evaluated as potential sanitation treatments in laboratory studies. In *in vitro* studies on the effect of spore viability, acidified hydrogen peroxide was more effective against *R. stolonifer* than against *B. cinerea* or *P. expansum*. More than 90% of the spores were killed after a 4-min incubation at 50-ppm hydrogen peroxide. For *B. cinerea* and *P. expansum* rates of 100 ppm and an 8-min incubation time or a rate of 150 ppm and a 4-min incubation time were required to obtain a similar efficacy. When spores were incubated at the maximum registered rate of Captan (1200 ppm), 85.9% and 87.3% of the spores of *B. cinerea* were killed after 10 and 22 h, respectively. *P. expansum* was less sensitive and only 35.5% and 43.3% of the spores were not viable anymore after the respective treatment durations. These data indicate that Captan possibly could be used as a sanitation treatment in re-circulating fungicide solutions or in lignosulfate float tanks where long contact times can be provided without affecting the performance of the fungicide.

INTRODUCTION

Gray mold, caused by *Botrytis cinerea*, and blue mold, caused mainly by *Penicillium expansum* in addition to some 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. Gray mold infections generally start at the stem end that is cut at harvest and becomes contaminated by the

omnipresent spores of the pathogen. On Bartlett pears, calyx end-rot caused by *B. cinerea* is common that starts from infections during bloom. Additional entry points for all pathogens are wounds that are caused by abiotic or biotic agents before or during harvest. While some postharvest decay fungi like *Rhizopus* species are suppressed at storage temperatures of 0°C (32°F), *B. cinerea* and *P. expansum* will still grow, although slowly. Thus, additional chemical treatments are needed. Our preharvest studies with ziram and captan gave inconsistent and generally unsatisfactory results as stand-alone treatments for postharvest decay management. Until the recent registration of Penbotec and Scholar, only thiabendazole (Mertect 340F) and captan (Captan 50WP) were available for postharvest use on pears. New fungicides were developed by others and us because resistance against TBZ is wide-spread among the pome fruit pathogens *B. cinerea* and *P. expansum*. Additionally, captan applied at the registered postharvest rate of 2 lb/200,000 lb is not very effective in reducing the incidence of decay. Furthermore, export restrictions of captan in different international markets exist and visible residues of the fungicide formulation are left on the fruit after treatment. The fungicide, however, may be effective as a direct contact fungicide. Thus, postharvest alternatives were needed. In our evaluations the biological control agent Bio-Save was inconsistent and was never as effective as the fungicides Elevate, Scholar, or Penbotec. The latter two fungicides were registered in California in 2005. Both fungicides are effective against TBZ-resistant isolates of *B. cinerea* and *P. expansum*.

The efficacy of Elevate (fenhexamid) against gray mold and of Scholar (fludioxonil), Pristine (boscalid/pyraclostrobin) and Penbotec (pyrimethanil) against gray mold and blue mold has been demonstrated in our studies that were summarized in our previous years' Annual Reports for the California Pear Board. All fungicides belong to different classes and they are classified as 'reduced-risk' by the US-EPA. In 2006, additional studies were conducted on the efficacy of Scholar and Penbotec on Bartlett and Bosc pears on experimental and commercial packinglines. We evaluated the efficacy of different application methods and the performance of these fungicides in mixtures with TBZ, captan, and chlorine. Mixtures of TBZ with the new fungicides were evaluated as a strategy to reduce the potential of resistance development against the new fungicides and to slow the build-up of TBZ-resistant populations of the pathogens. Captan and chlorine were evaluated as mixing partners for the new fungicides because of their potential as sanitation agents in re-circulating fungicide solutions and in float tanks (captan only). Thus, our goal is to have several new fungicides with different modes of action registered for postharvest use on pear available to be able to design resistance management strategies with fungicide mixtures and fungicide rotations and to optimize fungicide application methods to improve fungicide efficacy and efficiency. Additional studies conducted in 2006 focused on the evaluation of preharvest treatments for postharvest decay control.

OBJECTIVES

1) Evaluate of pre-harvest (fenhexamid - Elevate) and postharvest (fenhexamid - Elevate, fludioxonil - Scholar, and pyrimethanil - Penbotec) reduced-risk fungicides for postharvest management of gray mold and blue mold. TBZ-sensitive, and -resistant isolates of the pathogens will be used in inoculations and natural incidence of decay will be evaluated.
    i. Preharvest treatments with Elevate for postharvest decay control.
    ii. Experimental packing line treatments with postharvest fungicides.
    iii. Large-scale packinghouse studies with postharvest fungicides.
2) Evaluation of captan, chlorine, and acidified hydrogen peroxide as sanitizers of fungicide drench solutions or other water-tank systems (e.g., float tanks).
3) Continue to conduct pathogen population studies to determine baseline fungicide sensitivity levels in selected commercial packinghouses and monitor the presence of TBZ-resistance.
4) Identify species of *Penicillium* causing decay of pears using our newly developed molecular techniques.
5) Evaluate the new biocontrol Arabesque as a biofumigant for management of postharvest decays.

MATERIALS AND METHODS

Evaluate preharvest applications of new fungicides for postharvest disease management. Preharvest applications were evaluated on Bosc (2005-06) and Shingo (2006-07) pear fruit in commercial orchards.

Fungicides and their rates that were evaluated include Scala 600SC (pyrimethanil – 18 fl oz/A), Vangard 75WG (cyprodinil - 10 oz/A), Pristine 38WG (boscalid-pyraclostrobin - 0.92 lb/A), Elevate 50WDG (fenhexamid – 1.5 lb/A), and Evito 480SC (fluoxystrobin 10 fl oz/A). Applications were done at 7, 7 and 1, or 1 day PHI. Four replications of each fungicide were applied in a completely randomized design using an air-blast sprayer (100 gal/A). To evaluate the efficacy of preharvest treatments for control of natural incidence of postharvest fruit decay, ca. 100 harvested fruit per replication were dumped into pear float tanks in a packinghouse, rinsed with water, and stored at 1 C under commercial conditions for 6 months.

Efficacy of postharvest treatments using single fungicides and mixtures. Scholar 230SC and Penbotec 400SC were evaluated alone or in mixtures with Mertect 340F or Captan 50WP. Bartlett or Bosc pears were wound-inoculated with TBZ-resistant isolates of *B. cinerea* or *P. expansum*, incubated for 10-16 h, and then treated with fungicides. Fungicides were applied on an experimental or commercial packingline as aqueous solutions or in a diluted carnauba fruit coating using a low-volume spray system. In in-line drench or dip applications, fungicides were applied as aqueous solutions. After treatment, fruit were stored at 20 C, 95% RH for 6 to 8 days and then evaluated for decay. In delayed treatments, fruit were inoculated and incubated for 7 or 21 days at ca. 2 C before in-line drench applications were done. Data were analyzed using analysis of variance and averages were separated using least significant difference mean separation procedures of SAS 9.1.

Evaluation of acidified hydrogen peroxide as a new sanitation treatment to reduce the amount of viable spore inoculum in solutions. In laboratory studies to evaluate the in vitro activity of acidified hydrogen peroxide (provided as the product Perasan) spores of *B. cinerea, P. expansum*, or *R. stolonifer* were exposed to the chemical at selected concentrations. After selected incubation times, solutions were diluted 50 times and aliquots were plated onto agar media to quantify spore survival based on colony formation.

Evaluation of the stability of fungicides in chlorine-fungicide mixtures used as postharvest fungicide treatments. Laboratory studies were conducted using fungicides or fungicide-100 ppm chlorine solutions that were prepared 16 h before use and then used to treat pre-inoculated and incubated fruit. For this, fruit were wound-inoculated with *B. cinerea* (10^5 spores/ml) or *P. expansum* (10^6 spores/ml). After 12 h fruit were dipped for 30 sec into fungicide or fungicide-chlorine solutions. Fruit were then incubated at 20C for 6 days. In a similar experimental packingline study, using freshly prepared fungicide-chlorine mixtures, fungicides were applied as drench applications to wound-inoculated fruit that were incubated for 14-16 h prior to treatment. The performance of the fungicides was evaluated by determining the incidence of decay using 4 replications of 6 fruit in the laboratory study and 4 replications of 18 fruit in the packingline study. Data were analyzed using analysis of variance and averages were separated using least significant difference mean separation procedures of SAS 9.1.

Evaluation of captan as a sanitation treatment to reduce the amount of viable spore inoculum in *in vitro* exposure studies. In laboratory studies, conidia of *B. cinerea* or *P. expansum* were incubated in 1200-ppm solutions of captan for selected time periods. Control treatments received water instead of the fungicide. Incubation mixtures were then diluted 50 times with sterile water, and an aliquot of the solutions was plated out onto potato dextrose agar. Emerging fungal colonies were counted for up to three days. Development of fungal colonies was used as an indicator for spore survival, and relative survival in the treatments was calculated based on the number of colonies in the control.

Evaluation of chlorine or captan as a sanitation treatment to reduce the amount of viable spore inoculum in aqueous or lignosulfate solutions. Laboratory studies were conducted using aqueous preparations of fungicides (Scholar or Penbotec prepared alone or mixed with captan) or fungicide-100 ppm chlorine solutions. To each solution, pathogen spores (4×10^3 for *B. cinerea* or 8×10^4 for *P. expansum*) were added and the preparation was incubated for 16 h at 25C. Pathogen-spore survival was determined by dipping wounded Bartlett and Bosc fruit into each solution and incubating fruit for the development of decay for 6 days at 25C. Development of decay was used as an indicator for spore survival. Rhizopus rot developed as natural incidence of decay. Four replications of 18 fruit for each Bosc and Bartlett pears were used in this laboratory study.

In additional experiments, spores of the postharvest pathogens and captan were added to water or lignosulfate solutions. Wounded fruit were dipped in these mixtures for 1 min and then incubated at 20 C. Control solutions consisted of water or lignosulfate plus fungal spores. Development of decay was used as an indicator for spore survival. Aliquots of the mixtures were also plated out on agar in order to quantify the number of viable fungal propagules. Twelve fruit with three wounds each were used in each of four replications. Data were analyzed using analysis of variance and averages were separated using least significant difference mean separation procedures of SAS 9.1.

RESULTS AND DISCUSSION OF 2006 RESEARCH

Evaluation of preharvest fungicide applications for postharvest decay control. Preharvest treatments for control of natural incidence of postharvest decays were conducted on Bosc pears. Results from the first study are from the 2005-06 trial where fruit were incubated for 6 months under commercial conditions. The incidence of blue mold was very low in this experiment and no data could be obtained. For gray mold, 13.6% of the control fruit developed decay (Fig. 1). All treatments significantly reduced the incidence of decay. The most effective treatments were Elevate and the strobilurin fungicide Crown (both 0% decay) as well as Pristine (1.5% decay). The least effective treatments were Vangard (9% decay) and Scala (4.7% decay). Fruit from the second trial that was conducted in 2006 are still being incubated and thus, data are pending. Our studies over several years indicate that the efficacy of preharvest fungicide treatments of pears is not consistent and generally is not as high as the use of postharvest treatments. They still, however, can be part of an integrated management program.

Efficacy of postharvest treatments using single fungicides or mixtures. In experimental packingline studies using low-volume spray applications with Scholar, Mertect, Captan, and mixtures of Scholar with Mertect or Captan, similar results were obtained for Bosc and Bartlett pears that were wound-inoculated with TBZ-resistant isolates of the pathogens (Fig. 2). Treatments that contained Scholar (with or without the addition of Mertect or Captan) at a rate of 225-300 ppm were the most effective. Incidence of gray mold was $\leq$6.3% and for blue mold it was $\leq$12.5% as compared to 100% incidence in the control. The lower rate of Scholar (150 ppm) was numerically, but not always statistically, less effective. Mixtures of Scholar and Captan or Scholar and Mertect were similarly effective than Scholar alone. Mertect by itself reduced the incidence of gray mold by ca. 50% and the incidence of blue mold only by up to 20%. Captan was more effective against gray mold ($\leq$ 65% reduction in decay) than against blue mold ($\leq$35% reduction in decay).

In a commercial packingline study with Penbotec and Penbotec-Captan mixtures the efficacy of low-volume spray (31 gal/200,000 lb fruit) and in-line drench applications was compared on Bartlett and Bosc pears. Captan alone (used as a dip treatment) did not reduce the incidence of gray mold or blue mold as compared to the control (Fig. 3). The addition of Captan to Penbotec, however, statistically reduced the incidence of blue mold in most cases as compared to using Penbotec by itself, whereas for gray mold there was no effect. In comparing the two application methods, there was no consistent difference between the methods. Application methods were also compared in an experimental packingline study using Bartlett and Bosc pears. Overall, treatment efficacy was higher on the Bartlett than on the Bosc pears, presumably because the Bartlett pears were very ripe when the experiment was conducted and thus, were more susceptible to decay (Fig. 4). For both Scholar and Penbotec significantly less gray mold and blue mold decay developed when fruit were treated using in-line drenches as compared to low-volume spray applications. These results are consistent with our previous years' data where we demonstrated the higher efficacy of in-line drench applications as compared to low-volume spray inoculations. An explanation for not finding the same trend in the commercial packingline study (Fig. 3) is that in this latter study the in-line drench system was not set up properly. The treatment bed was very short and thus, the pass time of the fruit through the drencher may not have been long enough. Rhizopus rot developed as a natural incidence in this study on the control fruit and after Penbotec treatments, but not after treatments with Scholar.

Delayed fungicide treatments were conducted where fruit were wound-inoculated, stored at ca. 2 C for 7 or 21 days, and then treated using in-line drench applications with Scholar or Penbotec. When fruit were treated after 7 days (no visible signs of decay development), both fungicides were still very effective (Fig. 5A). No decay developed after treatment with Scholar, whereas 9.8% and 4.5% incidence of gray mold and blue mold, respectively, were observed after treatment with Penbotec. When fruit were stored for 21 days before treatments, decay caused by these low-temperature tolerant pathogens had already started to develop. Thus, treatments were evaluated for their effect on pathogen sporulation on the decaying fruit. The amount of sporulation was reduced by both fungicides for gray mold and blue mold, and less sporulation was observed for Scholar than for Penbotec (Fig. 5B). Using a sporulation rating scale from 0-4, ratings of 3.85 and 3.72 were observed on the untreated controls for gray mold and blue mold, respectively. These ratings were 0.93 and 0.81, respectively, for Scholar, and 2.61 and 2.5, respectively, for Penbotec.

Evaluation of acidified hydrogen peroxide as a new sanitation treatment to reduce the amount of viable spore inoculum in solutions. Acidified hydrogen peroxide (used as the product Perasan) was evaluated as a potential sanitation treatment in laboratory studies. In in vitro studies on the effect of spore viability, acidified hydrogen peroxide was more effective against *R. stolonifer* than against *B. cinerea* or *P. expansum*. More than 90% of the spores were killed after a 4-min incubation at 50 ppm hydrogen peroxide (Fig. 6). For *B. cinerea* and *P. expansum* rates of 100 ppm and an 8-min incubation time or a rate of 150 ppm and a 4-min incubation time were required to obtain a similar efficacy. Thus, Perasan could be an effective sanitation treatment, however, the rates required ($\geq$100 ppm) may not be economical.

Evaluation of the stability of fungicides in chlorine-fungicide mixtures used as postharvest fungicide treatments. When wound-inoculated fruit were dipped into 16-h old Scholar- or Penbotec-chlorine solutions, decays were reduced to incidences of 5.6% or less as compared to almost 100% decay incidence in the control (Fig. 7). An experimental packingline study was done to evaluate the effect of sodium hypochlorite (100 ppm chlorine) on efficacy of Scholar and Penbotec on Bartlett and Bosc pears. Chlorine by itself did not have any effect in reducing the incidence of gray mold or blue mold of wound-inoculated fruit (Fig. 8). Scholar at rates between 150 and 600 ppm reduced the incidence of gray mold to 0-14.3% (as compared to 100% in the control) and of blue mold to 0-21.4% (as compared to 100% in the control). The addition of chlorine to Scholar numerically, and sometimes significantly, reduced the incidence of both decays. Similar results were obtained for Penbotec where 2.8-4.8% and 0-23.7% decay was decay was obtained for gray mold and blue mold, respectively, when using the fungicide by itself (Fig. 8). Again, the addition of chlorine numerically, and sometimes statistically, reduced the incidence of decay.

These studies indicate that both Scholar and Penbotec were still very effective after the chlorine exposure, but does not rule out a partial degradation of the fungicides. Previous studies by us had shown that Scholar (fludioxonil) is very stable in chlorine solutions, whereas Penbotec (pyrimethanil) is partially degraded. Thus, under less optimal treatment conditions a reduced efficacy of Penbotec might still be expected. In these experiments, Rhizopus rot developed as a natural incidence on the control fruit and after chlorine, Captan, and Penbotec treatments, but not after treatments with Scholar.

Evaluation of captan as a sanitation treatment to reduce the amount of viable spore inoculum in *in vitro* exposure studies. In laboratory studies where conidial suspensions of *B. cinerea* or *P. expansum* were exposed to Captan for 4, 10, and 22 h, 60.6%, 85.9%, and 87.3% of the *B. cinerea* spores were not found to be viable anymore as compared to 100% viability of the control spores. For *P. expansum*, 18.3%, 35.5%, and 43.3% of the spores were non-viable for the same incubation times, respectively. Thus, Captan is more effective against gray mold spores than against blue mold spores. Long exposure times were required. Still, for the potential use of Captan as a sanitation treatment for re-circulating fungicide solutions and for float tanks, this would not be a limiting factor considering that incubations would be continuous and include overnight exposures.

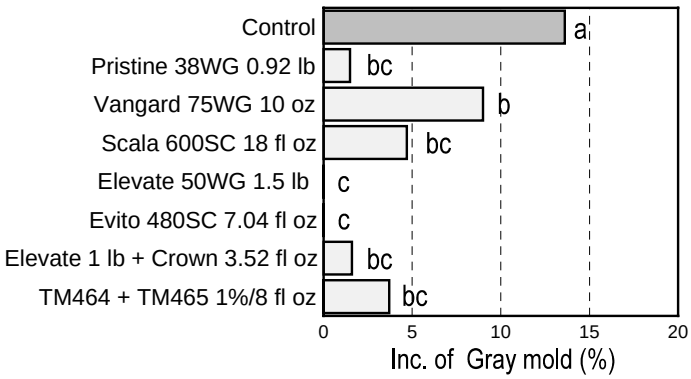
Evaluation of chlorine or captan as a sanitation treatment to reduce the amount of viable spore inoculum in aqueous or lignosulfate solutions. Laboratory studies were conducted using solutions of fungicides, fungicides with 100 ppm chlorine, and spores of each pathogen. Solutions were incubated for 16 h

before use. Pathogen-spore survival was determined by dipping wounded Bartlett and Bosc fruit into each solution and incubating fruit for the development of decay. No significant differences were found between the three rates of Scholar evaluated (150, 300, and 600 pm). Using both Scholar or Penbotec, decay was reduced to 5.6% or less for gray mold and blue mold as compared to $\geq$94.4% decay incidence in the controls (Fig. 9). The addition captan or chlorine to the solutions had no effect on the efficacy of both fungicides. Incubation of spores with chlorine alone also reduced the incidence of decay to very low levels, indicating that chlorine had killed most of the pathogen spores after the 16-h exposure. For Captan, only the incidence of gray mold was reduced to low levels, but not for blue mold. This indicates that Captan had killed most spores of *B. cinerea* and that the fungicide was much less effective against spores of *P. expansum*.

In dipping studies with wounded pear fruit where fungal spores and captan were added to water or lignosulfate, the addition of captan in most cases significantly reduced the incidence of gray mold and blue mold, indicating that some of the pathogen spores had been killed by the fungicide and thus, no longer could cause decay on the wounded fruit (Figs 10,11). Efficacy of captan was higher in the aqueous solutions (Fig. 11A) than in lignosulfate (Figs. 10A, 11A). These data are in agreement with the laboratory study in Fig. 9 where both decays were reduced when fungal spores were incubated in aqueous solutions of Captan for 16 h. The efficacy of Captan against blue mold, however, was much lower in the first study (Fig. 9), probably because a 2.7-time higher spore concentration of the fungus was used in this study as compared to the study in Fig. 11A.

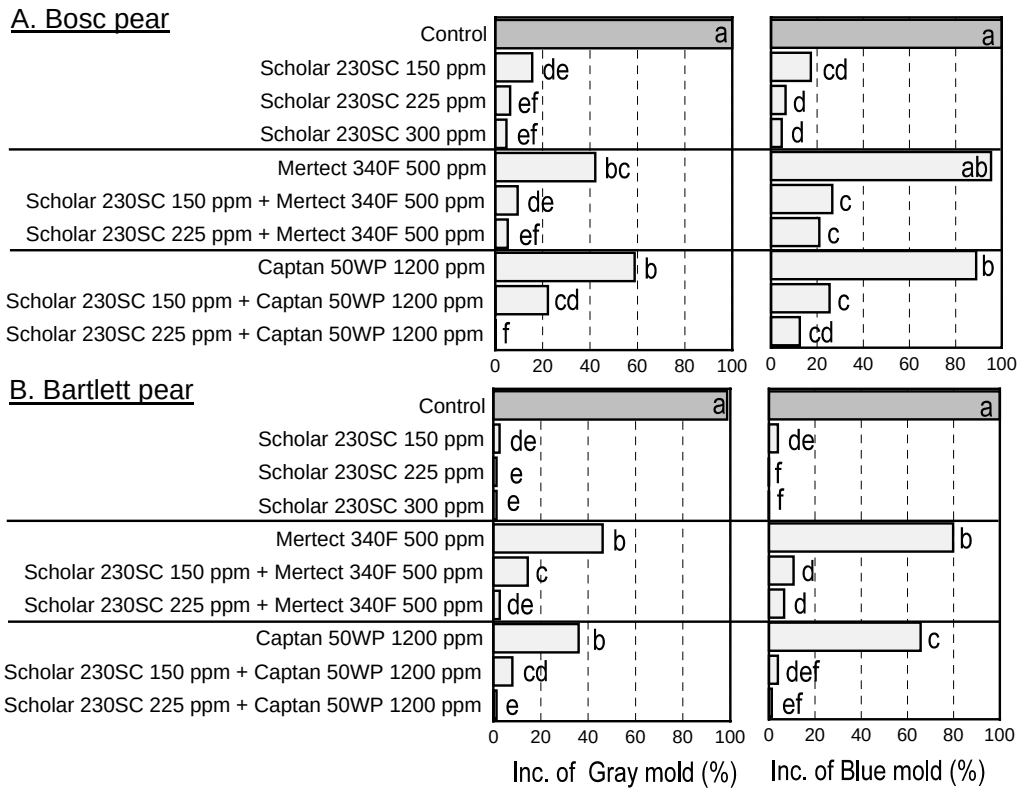
Lignosulfate solutions of the above study were also plated out on nutrient media to enumerate the number of microorganisms in the different treatments. Lignosulfate solutions contained a very large number of bacteria and yeast fungi, so that gray mold colonies could not be visualized. In both experiments the number of *P. expansum* colonies was significantly reduced after the addition of captan (Fig. 10B, 11B). Interestingly, the number of yeast and bacterial colonies was reduced from very high numbers (enumeration was not possible) in the control to very low numbers in the Captan treatment.

Fig. 1. Evaluation of 7+1 day preharvest treatments with fungicides for management of gray mold Bosc pears 2005-06



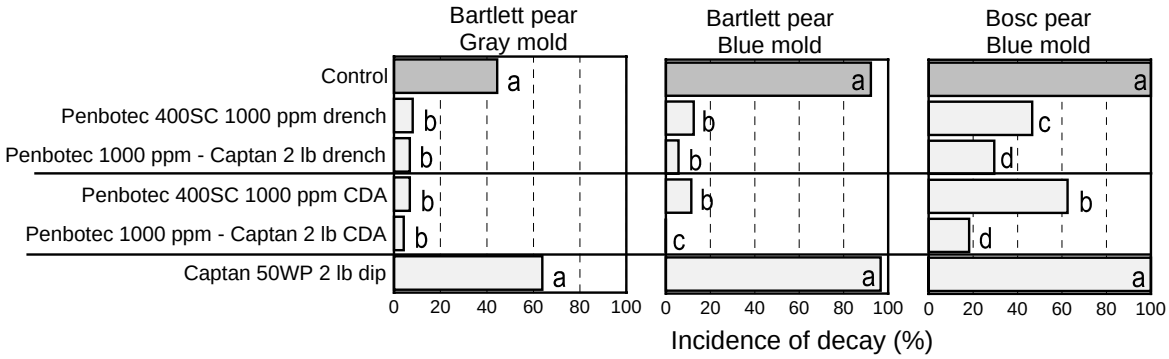
Applications were done in the field 7 and 1 days before harvest using an air-blast sprayer at a rate of 100 gal/A. Fruit were harvested, and stored in a packinghouse under commercial conditions for 6 months.

Fig. 2. Evaluation of postharvest treatments (CDA applications) with single fungicides and mixtures for management of postharvest decay of pears
- Experimental packingline study -



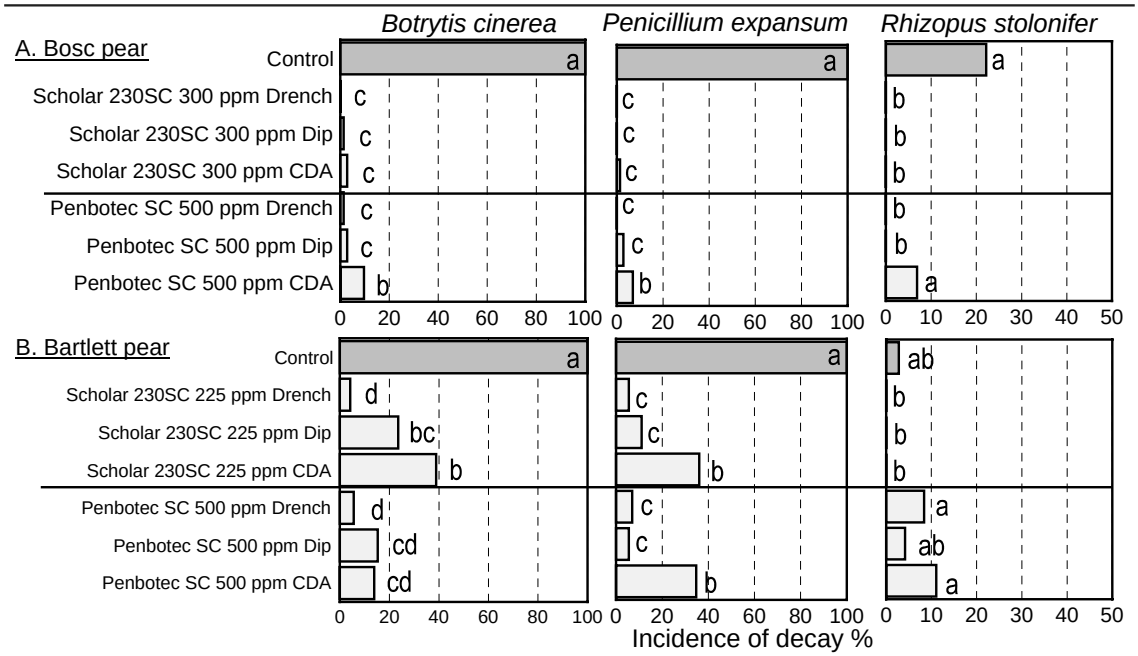
Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 10 h at 20C, treated using a CDA applicator at 25 gal/200,000 lb), and incubated again at 20C. All treatments were applied in 25% dilution of a carnauba fruit coating.

Fig. 3.. Evaluation of postharvest application methods using Penbotec and Captan for management of postharvest decay of pears
- Commercial packingline study -



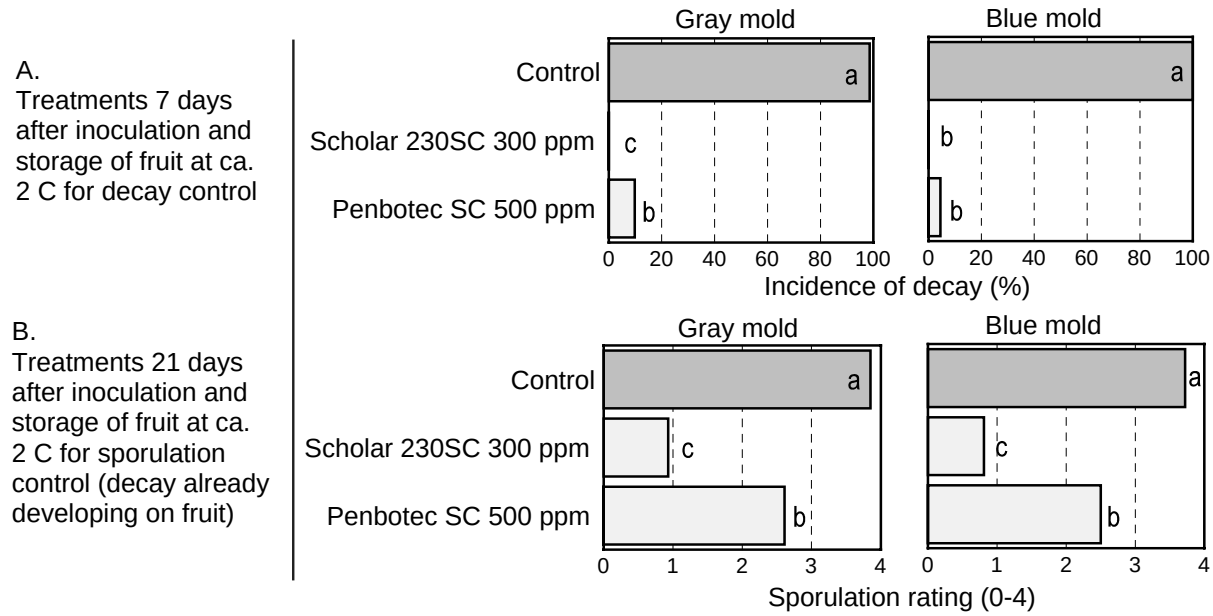
Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 3 h at 20C, and treated using a CDA applicator at 31 gal/200,000 lb) or an in-line drencher. All treatments were applied as aqueous solutions. Fruit were then incubated again at 15-20C.

Fig. 4. Evaluation of postharvest application methods using Scholar and Penbotec for management of postharvest decay of pears
- Experimental packingline study -



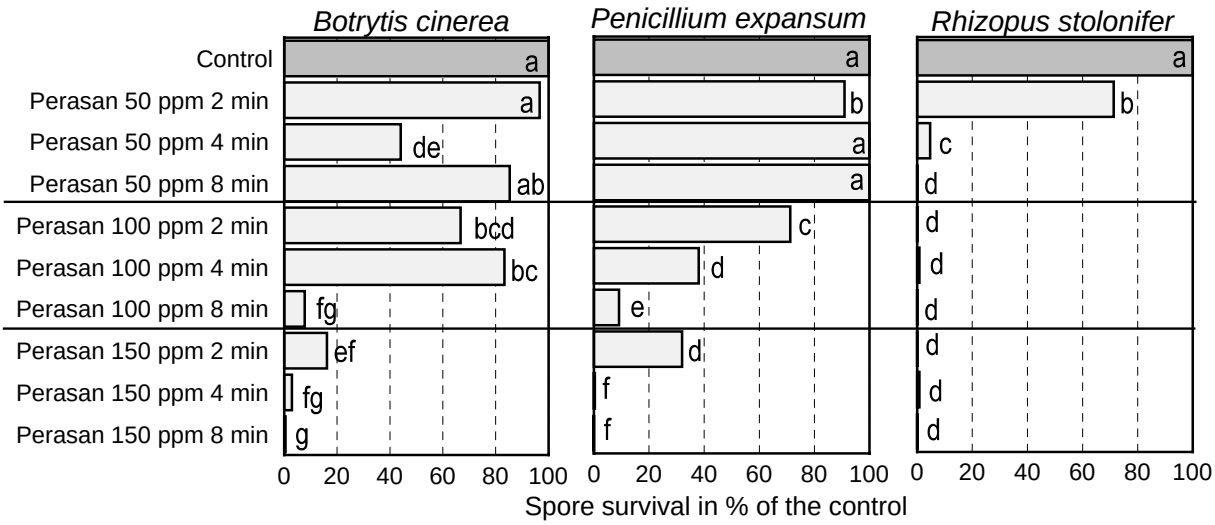
Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 13-16 h at 20C, and treated using an in-line drencher or a CDA applicator at 20 gal/200,000 lb, or by a 30-sec dip. All treatments were applied as aqueous solutions. Fruit were then incubated at 20C for 6 days. Rhizopus rot developed as natural incidence.

Fig. 5. Efficay of delayed fungicide in-line drench treatments for management of postharvest decays of Bosc pears in an experimental packingline study



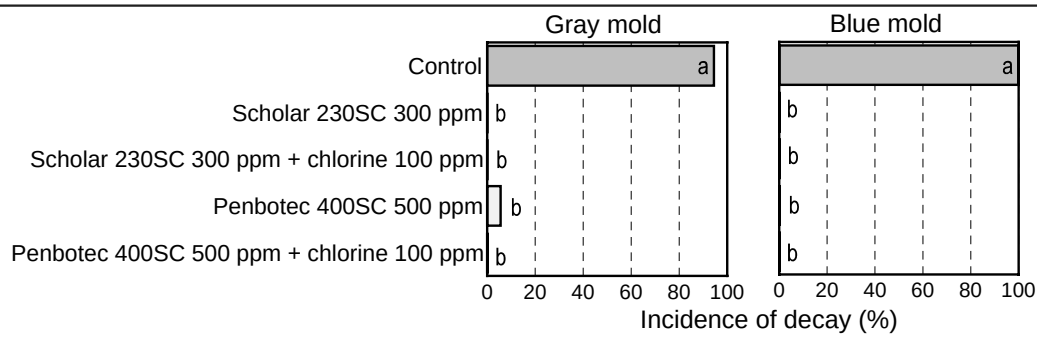
Fruit were wound-inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum* and incubated at ca. 2C. In-line drench treatments for decay control were done after 7 days. At the second treatment time, fruit already started developing decay. Thus, treatments were done to evaluate the effect of fungicides on sporulation of the pathogens. After treatment fruit were incubated for 6 days at 20C. All treatments were applied as aqueous solutions.

Fig. 6. Evaluation of sanitation treatments with hydrogen peroxide for their efficacy in killing spores of postharvest decay pathogens of pears in laboratory studies



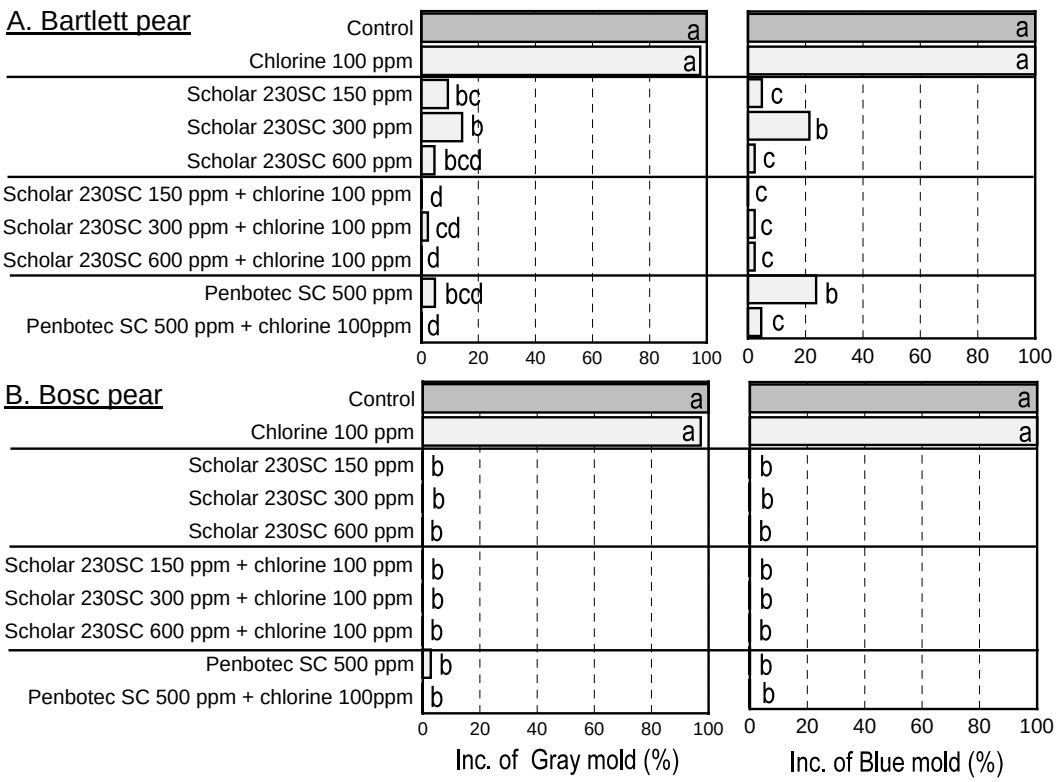
Fungal spores were incubated for 2, 4, or 8 min with 50, 100, or 150 ppm of hydrogen peroxide (used as Perasan). Mixtures were then diluted 1:50 with sterile water and aliquots were plateds out on PDA. Conony development was used as an indication of spore survival.

Fig. 7. Evaluation of mixtures of Scholar or Penbotec with chlorine for decay control on Bosc pear



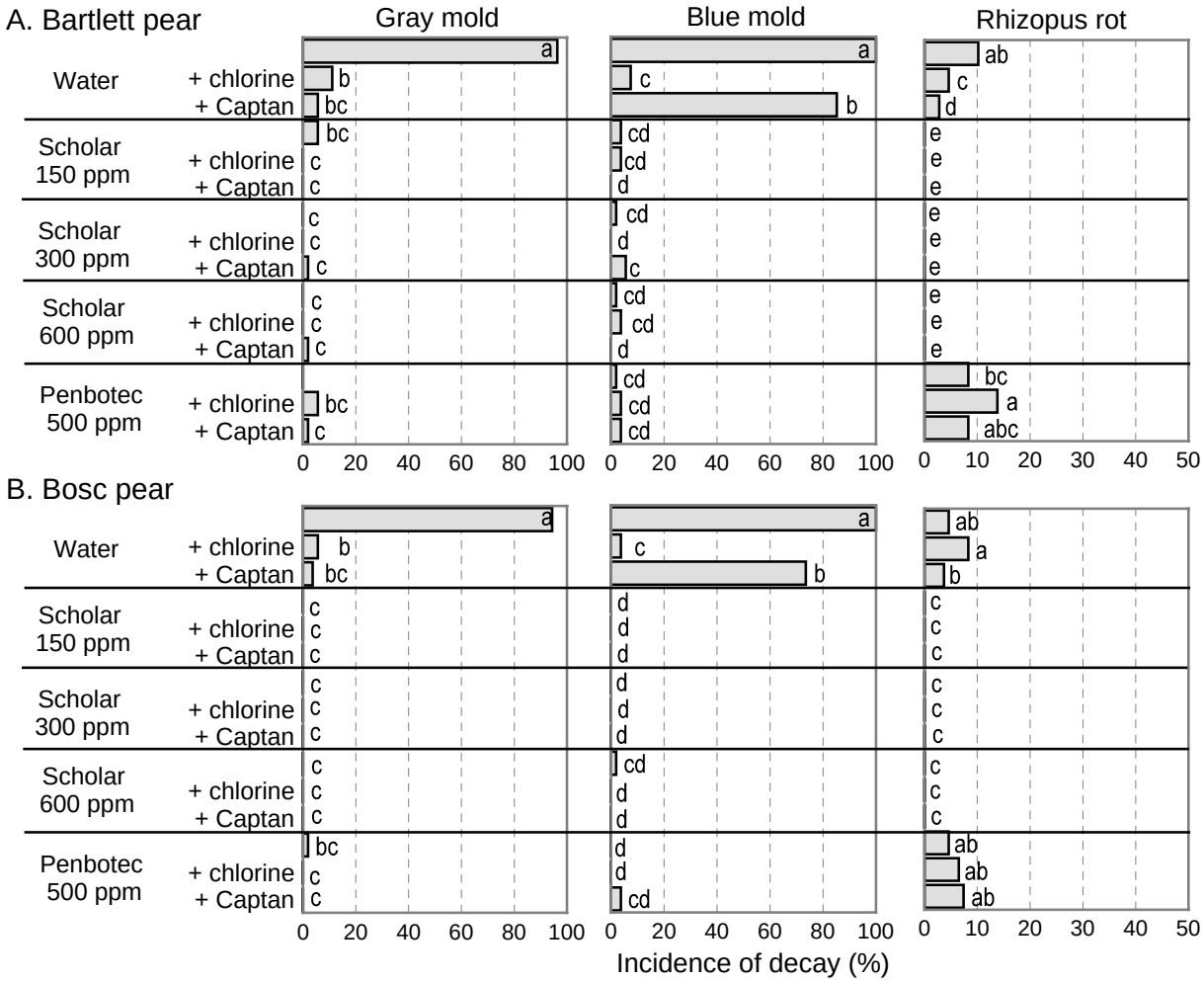
Fruit were wound-inoculated with *B. cinerea* (10^5 spores/ml) or *P. expansum* (10^6 spores/ml). After 12 h fruit were dipped for 30 sec into fungicide or fungicide-chlorine solutions that were prepared 16 h before use. Fruit were then incubated at 20C for 6 days.

Fig. 8. Evaluation of postharvest treatments (drench applications) with fungicides and fungicide-chlorine mixtures for management of postharvest decay of pears - Experimental packingline study -



Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 14-16 h at 20C, treated using an in-line drench application system, and incubated at 20C. All treatments were applied as aqueous solutions.

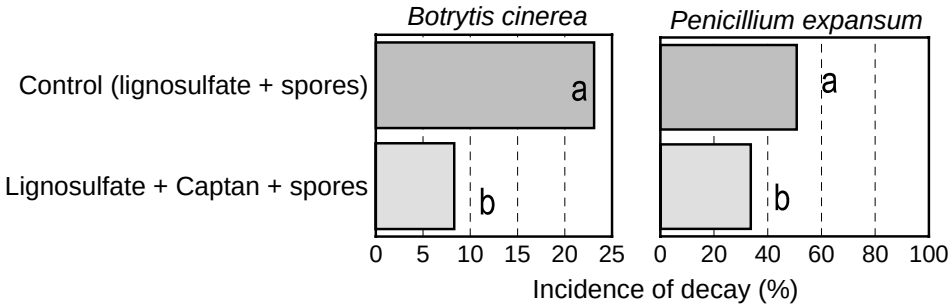
Fig. 9. Evaluation of the efficay of mixtures of Scholar or Penbotec with chlorine or captan in laboratory experiments



Solutions were prepared 16 h before use as following: to water first the fungicide (Scholar 230SC or Penbotec 400SC) was added, then spores of *B. cinerea* (final concentration 4 x 10^3 spores/ml) or *P. expansum* (final concentration 8 x 10^4 spores/ml), and then chlorine (100 ppm) or captan (2.5 lb /100 gal). Wounded fruit were dipped in solutions for 30 sec and then incubated at 20C for 6 days. Development of decay was used as an indicator for spore survival. Rhizopus rot developed as natural incidence of decay.

Fig. 10. Evaluation of Captan as a sanitation treatment of lignosulfate solutions

A. Decay development of fruit dipped in spore-Captan solutions



Fruit were wounded and dipped for 1 min in solutions of lignosulfate and spores of *B. cinerea* or *P. expansum* or solutions of lignisan, spores, and Captan (2 lb/100 gal). Fruit were then rinsed with water and incubated at 15-20C. Decay development on fruit was used as an indicator for spore survival.

B. Quantification of microorganisms in lignosulfate and lignosulfate-Captan solutions

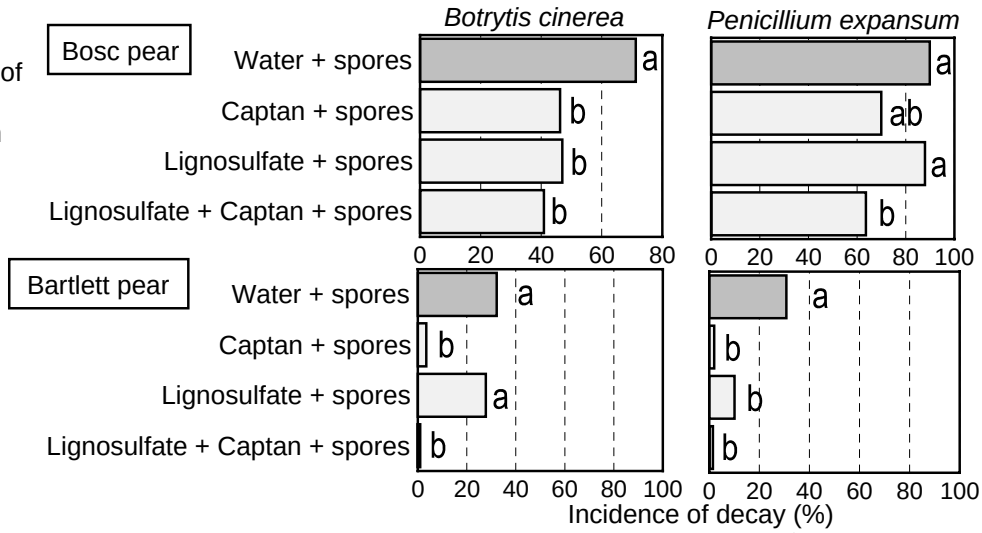
Colonies of microorganisms (cfu) per 0.1 ml*

Organism	Lignisan	Lignisan-Captan
P. expansum	120	8
Geotrichum sp.	120	16
Yeasts	>>**	3
Bacteria	>>>	>>

* - Solutions were plated out on agar media and colonies of microorganisms were quantified after 3 days of incubation at 24C.
** - High (>>) and very high (>>>) number of bacterial and yeast colonies.

Fig. 11. Evaluation of Captan as a sanitation treatment of water or lignosulfate solutions

A. Decay development of fruit dipped in spore-Captan solutions



Fruit were wounded and dipped for 1 min in solutions of lignosulfate and spores of *B. cinerea* (4×10^3/ml) or *P. expansum* (3×10^4/ml) or solutions of lignosulfate, spores, and Captan (2 lb/100 gal). Fruit were then rinsed with water and incubated at 15-20C. Decay development on fruit was used as an indicator for spore survival.

B. Quantification of microorganisms in lignosulfate and lignosulfate-Captan solutions

Colonies of microorganisms (cfu) per 0.1 ml*

Organism	Water	Water -Captan	Lignisan	Lignisan -Captan
P. expansum	160	78	60	0
Yeasts	0	0	>>>**	0
Bacteria	0	14	>>>	>>>

* - Solutions were plated out on agar media and colonies of microorganisms were quantified after 3 days of incubation at 24C.
** - High (>>) and very high (>>>) number of bacterial and yeast colonies.