FINAL PROJECT REPORT
WTFRC Project Number: CP08-802

Project Title: Control of postharvest fruit rots in apple

PI: Chang-Lin Xiao
Organization: WSU-TFREC, Wenatchee
Telephone: 509-663-8181 X229
Email: clxiao@wsu.edu
Address: 1100 N Western Ave
City: Wenatchee
State/Zip: WA 98801

Co-PI (2): Bruce Campbell
Organization: USDA ARS, Albany, CA
Telephone: 510-559-5846
Email: bcc@pw.usda.gov
Address: 800 Buchanan St.
City: Albany
State/Zip: CA 94710

Cooperators: Selected packinghouses across central Washington State

Other funding Sources: None

Total Project Funding: Year 1: $89,289 Year 2: $93,406

Budget History:

Item	2008	2009	2010 (extension)
Salaries[1]	54,331	56,001	
Benefits	21,173	23,505	
Wages (time slip)	5,000	5,000	
Benefits	785	900	
Equipment	0	0	
Supplies[2]	6,000	6,000	
Travel[3]	2,000	2,000	
Miscellaneous	0	0	
Total	89,289	93,406	0

Objectives:

1. Develop preharvest fungicides and postharvest fungicides integrated programs for decay control.
2. Develop preharvest fungicides and postharvest biocontrol integrated programs for decay control.
3. Develop pre- and post-storage integrated programs for decay control.
4. Determine patterns of sensitivity or resistance of fludioxonil- and pyrimethanil-resistant *Penicillium expansum* and *Botrytis cinerea* to various pre- and postharvest fungicides and use the information for guiding fungicide use.
5. Establish an industry-coordinated program to monitor the shift in sensitivity of *P. expansum* to fludioxonil and pyrimethanil.
6. Collaborate with Bruce Campbell in evaluating natural compounds for management of fungicide resistance and decay control.

Significant findings:

- Residual protection of apple fruit by preharvest Pristine was still evident five months after harvest but declined after the fruit were stored at room temperature for one week after cold storage.
- Preharvest Pristine plus postharvest BioSave further reduced blue mold incidence during cold storage. However, the effectiveness of these treatments declined after the fruit were stored at room temperature for one week after cold storage, suggesting that both Pristine and BioSave only suppress blue mold during cold storage.
- On Fuji and Red Delicious fruit, both Scholar and Penbotec on drenched fruit exhibited very good residual protection of fruit from infection by *Penicillium expansum*. BioSave alone applied at packing reduced blue mold incidence, but the effectiveness declined when the fruit were stored for one additional week at room temperature after cold storage. BioSave did not provide additional protection for Scholar-drenched or Penbotec-drenched fruit.
- All fludioxonil-resistant and pyrimethanil resistant mutants and wild-type isolates of *P. expansum* were sensitive to triflumizole (Procure, a DMI fungicide) and pyraclostrobin (a strobilurin fungicide). Pyrimethanil-resistant mutants were resistant to cyprodinil (Vangard), indicating that there is cross resistance between pyrimethanil and cyprodinil and that use of Vangard in the field could promote the development of resistance to pyrimethanil (Penbotec), a key postharvest fungicide.
- Pyrimethanil-resistant isolates of *Botrytis cinerea* were sensitive to fludioxonil, indicating no cross-resistance between pyrimethanil and fludioxonil. Pyrimethanil-resistant isolates were resistant to TBZ, but TBZ-resistant isolates that were sensitive to pyrimethanil also were observed among the isolates obtained from the same orchards where Topsin M and Vangard had been used in the past. The results indicate that there is no cross resistance between TBZ and pyrimethanil, but use of Topsin M and Vangard in these orchards resulted in *B. cinerea* isolates resistant to both TBZ and pyrimethanil. Development of *B. cinerea* populations resistant to TBZ and pyrimethanil in the field could compromise the efficacy of postharvest use of TBZ and pyrimethanil for gray mold control.
- In 2009, for the first time we detected pyrimethanil resistance in *P. expansum* obtained from decayed apple fruit from a packinghouse in which pyrimethanil had been used as a postharvest drench treatment in each of four consecutive years, suggesting that pyrimethanil-resistant individuals are emerging in *P. expansum* populations in Washington State after repeated use of pyrimethanil.
- Pyrimethanil applied at label rate completely controlled blue mold incited by a pyrimethanil-sensitive isolate, but 75% of the fruit that were inoculated with the pyrimethanil-resistant isolate and treated with pyrimethanil developed blue mold, indicating that pyrimethanil resistance in *P.*

expansum reported in this study can result in failure of blue mold control in apples with pyrimethanil.

- The finding of the occurrence of pyrimethanil resistance in *P. expansum* suggests that further research is needed to monitor the frequency of pyrimethanil-resistant populations, understand the biological characteristics of pyrimethanil resistance in *P. expansum*, determine whether the level of pyrimethanil resistance results in the failure of blue mold control with Penbotec, and develop relevant measures to manage pyrimethanil resistance.
- Octylgallate alone, or in combination with Scholar, did not control blue mold on apple fruit caused by fludioxonil-resistant *P. expansum*.

Methods:

Pristine was applied to Fuji fruit one week before harvest. Fruit were stored in CA for five months. Fruit were removed from CA, washed and brushed through a research packing line, wounded and inoculated with *P. expansum*, and treated with either BioSave or a fungicide. Inoculated fruit were stored at 32°F in air for eight weeks and one additional week at room temperature. Decay was evaluated.

Fruit of Red Delicious and Fuji from commercial orchards were drenched with either Scholar or Penbotec and stored in CA. The fruit were removed from CA five and seven months after harvest, washed and brushed through a research packing line, wounded and inoculated with *P. expansum,* and treated with either BioSave or a fungicide. Inoculated fruit were stored at 32°F in air for eight weeks and one additional week at room temperature. Decay was evaluated.

Blue mold-decayed fruit were collected from grower lots that had been drenched with Penbotec or Scholar from packinghouses. Isolations of *P. expansum* from decayed fruit were attempted. Isolates of *Penicillium* spp. were identified to species. Isolates of *P. expansum* were screened for resistance to pyrimethanil, fludioxonil and thiabendazole (TBZ). A subset of isolates was also tested to determine EC_{50} values of the fungicides.

Identified natural compounds from Bruce Campbell's lab were tested for activity against fungicide-resistant strains of *P. expansum* in an agar medium and apple fruit.

Results & Discussion:

Preharvest Pristine in combination with postharvest biocontrol agent or fungicide for blue mold control.

This experiment was conducted on Fuji in both 2008-09 and 2009-10 seasons. Pristine was applied to Fuji apples seven days before harvest. Fruit were removed from CA five months after harvest, washed and brushed through a research packing line, wounded and inoculated with *P. expansum,* and treated with either BioSave or a fungicide. Inoculated fruit were stored at 32°F in air for eight weeks and one additional week at room temperature. The results from the 2008-09 trial were reported last year. The results from 2009-10 season are summarized in Table 1. The results from the two seasons were consistent.

Residual protection of apple fruit by Pristine was still evident five months after harvest but declined significantly after the fruit were stored at room temperature for one week after CA storage (Table 1). The loss of residual protection by Pristine at room temperature was likely due to that Pristine only suppresses but not eradicate *P. expansum*. At room temperature, Pristine residues may decline, thus *P. expansum* resumes growth. BioSave alone reduced blue mold incidence to approximately 38% during the eight-week cold storage. Preharvest Pristine plus postharvest BioSave further reduced blue mold incidence to approximately 3% during cold storage. However, the effectiveness of these treatments declined after the fruit were stored at room temperature for one week after cold storage.

Table 1. Preharvest Pristine in combination with postharvest BioSave for blue mold control on Fuji apples in 2009-10 season.

Preharvest Treatment	Fungicide applied 5 months after harvest	8 weeks at 32F post inoculation		1 week at room temp after cold storage	
		% decay	Lesion (mm)	% decay	Lesion (mm)
Nontreated	No Fungicide	100.0a[z]	36.0a	100.0a	72.7a
	Scholar	0.0f	0.0d	0.0c	0.0d
	Penbotec	0.0f	0.0d	1.3c	13.0c
	TBZ	100a	32.7a	100a	70.5a
	BioSave	37.5d	11.9b	98.8a	37.5b
Pristine	No Fungicide	53.8c	12.3b	98.8a	37.6b
	Scholar	0.0f	0.0d	0.0c	0.0d
	Penbotec	0.0f	0.0d	0.0c	0.0d
	TBZ	76.3b	13.2b	100a	39.7b
	BioSave	2.5e	6.9c	78.8b	12.3c

[z] Values within the same column followed by the same letter are not significantly different according to the Waller-Duncan K-ratio *t* test at K ratio = 100 (*P* = 0.05).

Pre-storage fungicide drench in combination with postharvest biocontrol agent or fungicide for blue mold control.

Two trials (one on Fuji and one on Red Delicious) were conducted on the 2008 crops and results were presented in the 2009 research report. We repeated the two trials on the 2009 crops and finished in spring 2010. The results are presented in Table 2 and Table 3. On Fuji, both Scholar and Penbotec on drenched fruit showed very good residual protection of fruit from infection by *P. expansum* (Table 2). BioSave alone applied at packing reduced blue mold incidence to 33-43% on the 2008 crops during cold storage but not on the 2009 crops (Table 2). The effectiveness of BioSave was lost when the fruit were stored for one additional week at room temperature after cold storage. BioSave did not provide additional protection for Scholar-drenched or Penbotec-drenched fruit. The results suggest that Scholar and Penbotec have long lasting residual protections against *P. expansum* on drenched apple fruit.

Table 2. Postharvest drench in combination with BioSave applied at packing for blue mold control on Fuji apples in 2009-10 season.

Drench treatment applied prior to storage	Fungicides applied at packing 5 or 7 months post drenching	5 months post drench treatments: % blue mold at 8 weeks at 0ºC post packing	5 months post drench treatments: % blue mold at one additional week at room temperature after storage	7 months post drench treatments: % blue mold at 8 weeks at 0ºC post packing	7 months post drench treatments: % blue mold at one additional week at room temperature after storage
Nontreated	No fungicide	95.0	97.5	100.0	100.0
	Scholar	0.0	0.0	0.0	0.0
	Penbotec	0.0	0.0	0.0	0.0
	TBZ	2.5	8.8	0.0	5.0
	Bio-Save	90.0	95.0	95.0	100.0
Scholar	No fungicide	0.0	1.3	0.0	8.8
	Bio-Save	0.0	1.3	1.3	7.5
Penbotec	No fungicide	0.0	0.0	0.0	2.5
	Bio-Save	0.0	0.0	0.0	0.0

On Red Delicious, both Scholar and Penbotec on drenched fruit also showed very good residual protection of fruit from infection by *P. expansum* during the eight-week cold storage, but Scholar's residual protection declined slightly when the fruit were move to room temperature (Table 3). BioSave alone applied at packing reduced blue mold incidence to 44-76%, but the effectiveness was lost when the fruit were stored for one additional week at room temperature after cold storage. BioSave did not provide additional protection for Scholar-drenched or Penbotec-drenched fruit. The results suggest that residues of Scholar and Penbotec on drenched Red Delicious apple fruit protect apple fruit for several months post-drenching.

Residual effects of Scholar and Penbotec on control of blue mold in Fuji apple fruit.

Previously we documented that when Scholar and Penbotec are applied to Red Delicious fruit as a postharvest drench treatment, they can provide long protection of the fruit from infection by *P. expansum*. In this project, we conducted similar trials on Fuji to examine whether this also occurs on Fuji apples. The results have been reported. In summary, when Fuji fruit were drenched with Scholar or Penbotec, excellent residual protection against *P. expansum* was still evident five and seven months after harvest. These results are consistent with what we observed on Red Delicious. Taken these together, our research suggests that residues of fludioxonil and pyrimethanil on/in apple fruit are persistent during cold storage and that residual protection of apple fruit by the two fungicides can last for at least seven months under apple-storage conditions.

Table 3. Postharvest fungicide drench in combination with BioSave applied at packing for blue mold control on Red Delicious apples in 2009-10 season

		5 months post drench treatments		7 months post drench treatments	
Drench treatment applied prior to storage	Fungicides applied at packing 5 or 7 months post drenching	% blue mold at 8 weeks at 0ºC post packing	% blue mold at one additional week at room temperature after storage	% blue mold at 8 weeks at 0ºC post packing	% blue mold at one additional week at room temperature after storage
Nontreated	No fungicide	98.8	98.8	100.0	100.0
	Scholar	0.0	1.3	0.0	0.0
	Penbotec	0.0	0.0	0.0	0.0
	TBZ	10.0	17.5	0.0	2.5
	Bio-Save	43.8	98.8	76.3	100.0
Scholar	No fungicide	0.0	7.5	0.0	10.0
	Bio-Save	0.0	5.0	0.0	12.5
Penbotec	No fungicide	0.0	0.0	0.0	0.0
	Bio-Save	0.0	0.0	0.0	0.0

Patterns of cross resistance or multi-drug resistance in pyrimethanil-resistant and fludioxonil-resistant mutants of Penicillium expansum and Botrytis cinerea.

All fludioxonil-resistant and pyrimethanil-resistant mutants and wild-type isolates of *P. expansum* were sensitive to triflumizole (Procure), a DMI fungicide (Table 4), indicating that the use of DMIs in the orchard likely will not increase the populations resistant to fludioxonil or pyrimethanil. Sensitivity of wild-type isolates and fungicide-resistant mutants to thiophanate-methyl (Topsin M) exhibited the same pattern of sensitivity to TBZ, indicating cross resistance between TBZ and Topsin M in fungicide-resistant mutants, including multi-drug resistance phenotypes. The four pyrimethanil-resistant mutants also were resistant to cyprodinil (Vangard), indicating the existence of cross resistance between pyrimethanil and cyprodinil (Table 4). Fludioxonil-resistant and pyrimethanil-resistant mutants and their parental wild-type isolates of *P. expansum* were sensitive to pyraclostrobin (a strobilurin fungicide) (data not shown).

We obtained 12 pyrimethanil-resistant *Botrytis cinerea* isolates from orchards where Vangard (cyprodinil) and Topsin M had been used in the past. Sensitivity of these isolates to selected fungicides was tested. All pyrimethanil-resistant isolates of *B. cinerea* were sensitive to fludioxonil, indicating no cross-resistance between pyrimethanil and fludioxonil (data not shown). All 12 pyrimethanil-resistant isolates were resistant to TBZ, but TBZ-resistant isolates that were sensitive to pyrimethanil also were observed among the isolates obtained from the same orchards. The results indicate that there is no cross resistance between TBZ and pyrimethanil, but use of Topsin M and Vangard in these orchards resulted in *B. cinerea* isolates resistant to both TBZ and pyrimethanil.

Table 4. In vitro sensitivity of mycelial growth of fludioxonil- and pyrimethanil-resistant mutants and their parental wild-type isolates of *Penicillium expansum* to triflumizole, thiophanate-methyl and cyprodinil.

Isolate	Phenotype	EC_{50} (mg/L) and phenotype: Triflumizole (DMI)		thiophanate-methyl (benzimidazole)		Cyprodinil (anilinopyrimidine)	
3354	TBZ^S Flu^S Pyr^S	0.121	S	3.211	S	0.353	S
3294	TBZ^{HR} Flu^S Pyr^S	0.175	S	> 200	HR	0.371	S
4277	TBZ^S Flu^{HR} Pyr^S	0.139	S	1.820	S	0.362	S
4284	TBZ^S Flu^{HR} Pyr^S	0.143	S	4.033	S	0.276	S
4262	TBZ^{HR} Flu^{HR} Pyr^S	0.238	S	> 200	HR	0.187	S
4272	TBZ^{HR} Flu^{HR} Pyr^S	0.252	S	> 200	HR	0.139	S
4256	TBZ^{LR} Flu^{LR} Pyr^R	0.290	S	18.990	LR	9.280	R
4258	TBZ^{LR} Flu^{LR} Pyr^R	0.330	S	16.940	LR	22.269	R
4252	TBZ^{HR} Flu^{LR} Pyr^R	0.290	S	> 200	HR	6.814	R
4253	TBZ^{HR} Flu^{LR} Pyr^R	0.293	S	> 200	HR	7.450	R

TBZ = thiabendazole, Flu = fludioxonil, Pyr = pyrimethanil, S = sensitive, R = resistant, LR = lowly resistant, HR = highly resistant.

Monitoring resistance of P. expansum to pyrimethanil and fludioxonil

Isolates of *P. expansum* were obtained from decayed apple fruit collected from packinghouses in 2008 and 2009. These isolates were tested for resistance to pyrimethanil, fludioxonil, and TBZ.

In 2008, The EC50 values of pyrimethanil for the isolates collected in 2008 ranged from 0.898 to 1.529 mg/L, with an average of 1.233 mg/L. The baseline EC50 values of pyrimethanil based on the 120 isolates collected in 2005 ranged from 0.519 to 2.054, with a mean of 1.340 mg/L. The results indicated that the sensitivity of these isolates to pyrimethanil remained at a similar level as the baseline population. EC50 values of fludioxonil for fungal mycelial growth ranged from 0.015 to 0.025 mg/L with an average of 0.021 mg/L. In comparison, the baseline EC50 values of fludioxonil based on the 120 isolates collected in 2005 ranged from 0.011 to 0.068 with an average of 0.020 mg/L. It appears that the sensitivity of these isolates to fludioxonil remained at a similar level as the baseline population.

In 2009, for the first time we detected pyrimethanil resistance in *P. expansum* obtained from decayed apple fruit. In total, 186 and 16 isolates of *P. expansum* were collected from Penbotec-drenched and Scholar-drenched apple fruit, respectively (Table 5). One isolate from Penbotec-drenched fruit showed significant resistance to pyrimethanil. EC_{50} values of pyrimethanil on mycelial growth and conidial germination for the resistant isolate were 9.9 and 3.1 µg/ml, respectively, which were 7.4-fold and 16.5-fold higher than the means of the baseline population. Whereas EC_{50} values of pyrimethanil for a subset of 37 pyrimethanil-sensitive isolates ranged from 0.632 to 1.518 mg/L, with a mean of 1.07 mg/L, which is within the baseline sensitivity.

All isolates were sensitive to fludioxonil. Of the 202 isolates tested, 35 were resistant to TBZ, indicating that TBZ-resistant strains remained in *P. expansum* populations even after TBZ was not used.

In a decay-control study, all inoculated fruit in the nontreated controls were decayed. Pyrimethanil applied at label rate completely controlled blue mold incited by a pyrimethanil-sensitive isolate, but 75% of the fruit that were inoculated with the pyrimethanil-resistant isolate and treated with pyrimethanil developed blue mold (Fig. 1). This is the first report of pyrimethanil resistance in *P. expansum* from decayed apple fruit collected from commercial packing houses. The pyrimethanil-resistant isolate was obtained from a packing house in which pyrimethanil had been used as a postharvest drench treatment in each of four consecutive years, suggesting that pyrimethanil-resistant individuals are emerging in *P. expansum* populations in Washington State after repeated use of pyrimethanil. Our results also indicate that pyrimethanil resistance in *P. expansum* reported in this study can result in failure of blue mold control in apples with pyrimethanil.

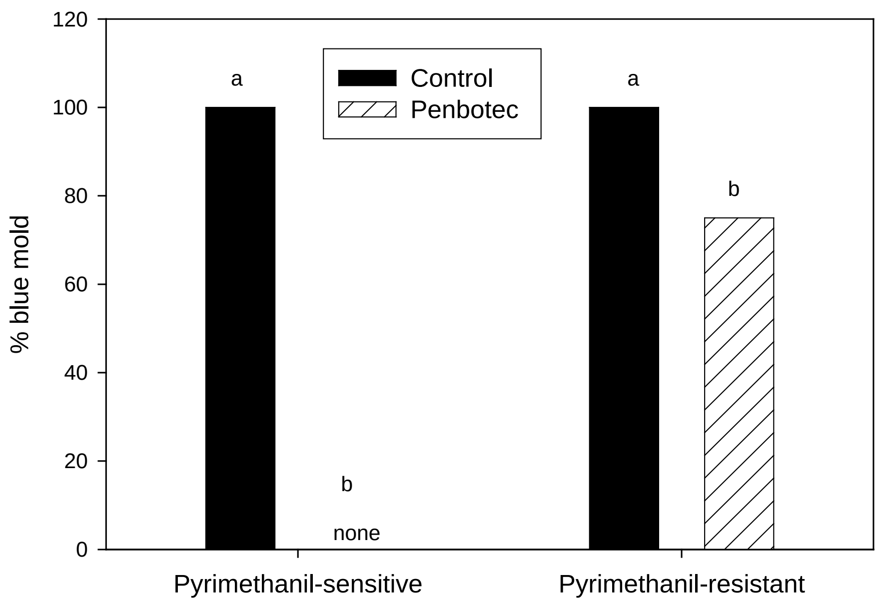

Fig. 1. Control of blue mold incited by pyrimethanil-sensitive and pyrimethanil-resistant strains of *Penicillium expansum* with Penbotec.

Table 5. Monitoring of resistance to postharvest fungicides in *Penicillium expansum* from apples

Source	**Drench Treatment**	**# isolates of *P. expansum***	**# isolates resistant to Penbotec**	**# isolates resistant to Scholar**	**# isolates resistant to TBZ**
Packinghouse 1	Penbotec	8	0	0	2
Packinghouse 2 –Lot 1	Penbotec	34	0	0	6
Packinghouse 3 –Lot 1	Penbotec	29	0	0	5
Packinghouse 3 –Lot 2	Penbotec	4	0	0	4
Packinghouse 3 –Lot 3	Penbotec	14	0	0	1
Packinghouse 3 –Lot 4	Penbotec	97	1	0	12
Packinghouse 2 –Lot 2	Scholar	16	0	0	5
Total isolates from Penbotec-drenched fruit		**186**	**1**	**0**	**30**

Evaluate natural compounds for controlling fludioxonil-resistant Penicillium expansum.

This research was done in collaboration with Bruce Campbell. In our previous study, we evaluated 2,5-DHBA 18 mM and 2,5-Dbald 1 mM as a chemosensitizing agent to overcome fludioxonil resistance of *Penicillium expansum* on apple fruit. We found that when used in combination with fludioxonil, these two compounds did not improve control of blue mold caused by a fludioxonil-resistant strain. To further explore the potential of using natural compounds to overcome fludioxonil resistance, Campbell lab did additional lab tests and found that on Petri dishes, octylgallate showed the potential as a promising chemosensitizing agent to overcome fludioxonil resistance in *P. expansum*. We further evaluated octylgallate in combination with Scholar (fludioxonil) for control of blue mold caused by two different fludioxonil-resistant strains (FR2: resistant to fludioxonil but sensitive to TBZ; FR3: resistant to both fludioxonil and TBZ) (Table 6). Octylgallate alone, or in combination with Scholar, did not control blue mold caused by fludioxonil-resistant *P. expansum*. It appears that in vitro and in vivo test results were not consistent. It is not known what causes the difference in results between the two tests, but compounds present in apple fruit flesh may affect the activity of octylgallate against fludioxonil-resistant *P. expansum*.

Table 6. Efficacy of octylgallate and Scholar for controlling blue mold caused by fludioxonil-resistant isolates (FR2 and FR3: fludioxonil resistant; W2: fludioxonil sensitive) of *Penicillium expansum*

Treatment	**% Blue mold 8 wk at 32 F**		**% Blue mold 1 week at room temperature**
	FR3	**W2**	**FR2**
CK	76.3	100	100
Octylgallate 0.15 mM	81.3	96.3	100
Octylgallate 1.0 mM	78.8	100	100
Octylgallate 0.15 mM + Scholar 230 SC	88.8	2.5	100
Octylgallate 1.0 mM + Scholar 230 SC	93.8	3.8	100
Scholar 230 SC	85	1.3	100

Executive Summary

This report is a summary of a two-year project conducted in 2008 and 2009. Part of the research was completed in 2010 because of the postharvest nature of the project. The goals of the project were to develop integrated programs using recently registered reduced-risk fungicides and a biocontrol agent to control major postharvest diseases in apples and to monitor and characterize resistance of *Penicillium expansum* to recently registered postharvest fungicides.

Blue mold caused by *Penicillium expansum* and gray mold caused by *Botrytis cinerea* are major postharvest diseases of apples. In the present project, we evaluated various pre- and postharvest integrated programs or pre- and post-storage integrated programs for decay control. Residual protection of apple fruit by preharvest Pristine was still evident 5 months after harvest but declined after the fruit were stored at room temperature for one week after cold storage. Preharvest Pristine in combination with postharvest biocontrol agent BioSave was more effective than Pristine alone in reducing blue mold at storage temperature. However, the effectiveness of these treatments declined after the fruit were stored at room temperature for one week after cold storage, suggesting that both Pristine and BioSave only suppress blue mold during cold storage.

When Penbotec and Scholar were applied as postharvest drench treatments prior to storage, the residues of these two fungicides seemed to be stable in treated Fuji fruit in CA storage conditions. This observation is similar to what we previously observed on Red Delicious. On Fuji and Red Delicious fruit, both Scholar and Penbotec on drenched fruit exhibited very good residual protection of fruit from infection by *Penicillium expansum*. BioSave alone applied at packing reduced blue mold incidence, but the effectiveness declined when the fruit were stored for one additional week at room temperature after cold storage. BioSave did not provide additional protection for Scholar-drenched or Penbotec-drenched fruit.

All fludioxonil-resistant and pyrimethanil resistant mutants and wild-type isolates of *P. expansum* were sensitive to triflumizole (Procure, a DMI fungicide) and pyraclostrobin (a strobilurin fungicide). Pyrimethanil-resistant mutants were resistant to cyprodinil (Vangard), indicating that there is cross resistance between pyrimethanil and cyprodinil and that use of Vangard in the field could promote the development of resistance to pyrimethanil (Penbotec), a key postharvest fungicide.

Pyrimethanil-resistant isolates of *Botrytis cinerea* were sensitive to fludioxonil, indicating no cross-resistance between pyrimethanil and fludioxonil. Pyrimethanil-resistant isolates were resistant to TBZ, but TBZ-resistant isolates that were sensitive to pyrimethanil also were observed among the isolates obtained from the same orchards where Topsin M and Vangard had been used in the past. The results indicate that there was no cross resistance between TBZ and pyrimethanil, but use of Topsin M and Vangard in these orchards resulted in *B. cinerea* isolates resistant to both TBZ and pyrimethanil. Development of *B. cinerea* populations resistant to TBZ and pyrimethanil in the field could compromise the efficacy of postharvest use of TBZ and pyrimethanil for gray mold control.

In 2009, for the first time we detected pyrimethanil resistance in *P. expansum* obtained from decayed apple fruit from a packinghouse in which pyrimethanil had been used as a postharvest drench treatment in each of four consecutive years, suggesting that pyrimethanil-resistant individuals are emerging in *P. expansum* populations in Washington State after repeated use of pyrimethanil. Pyrimethanil applied at label rate completely controlled blue mold incited by a pyrimethanil-sensitive isolate, but 75% of the fruit that were inoculated with the pyrimethanil-resistant isolate and treated with pyrimethanil developed blue mold, indicating that pyrimethanil resistance in *P. expansum* reported in this study can result in failure of blue mold control in apples with pyrimethanil. The finding of the occurrence of pyrimethanil resistance in *P. expansum* suggests that further research is needed to monitor the frequency of pyrimethanil-resistant populations, understand the biological characteristics of pyrimethanil resistance in *P. expansum*, determine whether the level of pyrimethanil resistance results in the failure of blue mold control with Penbotec, and develop relevant measures to manage pyrimethanil resistance.