FINAL PROJECT REPORT
WTFRC Project Number: CP-10-105

Project Title: Sustainable postharvest decay control

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Cooperators: Selected packinghouses across central Washington State

Total Project Funding: **Year 1:** $75,488 **Year 2:** $78,681

Other funding Sources:

Agency Name: Washington State Commission on Pesticide Registration
Amt. requested/awarded: $11,247

WTFRC Collaborative expenses:

Item	2010	2011
Stemilt RCA room rental	6,300	6,300
Crew labor	0	0
Shipping	0	0
Supplies	0	0
Travel	0	0
Plot Fees	0	0
Miscellaneous	0	0
Total	6,300	6,300

Budget History

Item	2010	2011	2012 (extension)
Salaries[1]	43,747	45,747	0
Benefits	17,149	18,550	0
Wages[2]	4,000	4,000	0
Benefits	592	384	0
Equipment	0	0	0
Supplies[3]	8,000	8,000	0
Travel[4]	2,000	2,000	0
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	75,488	78,681	0

Objectives:

1. Manage resistance to the postharvest fungicides pyrimethanil and fludioxonil in *Penicillium expansum.*
   a. Monitor and characterize resistance to pyrimethanil and fludioxonil in *P. expansum* populations.
   b. Develop fungicide programs for controlling blue mold caused by pyrimethanil-resistant *P. expansum.*
2. Manage resistance to Pristine in *Botrytis cinerea* and *Penicillium expansum.*
   a. Establish baseline sensitivity to Pristine in *P. expansum* populations.
   b. Monitor and characterize Pristine resistance in fungal pathogen populations.
   c. Develop fungicide programs for controlling gray mold and blue mold caused by Pristine-resistant strains.
3. Evaluate non-chemical approaches for postharvest decay control.

Significant Findings:

- Resistance to pyrimethanil (Penbotec) has developed in *Penicillium expansum* populations in some packinghouses where the fungicide as a postharvest drench has been used annually for 4-5 consecutive years. In one packinghouse, over 90% of the isolates were resistant to pyrimethanil when Penbotec (pyrimethanil) was again used on 2010 crops, while on the fruit drenched with Scholar in 2010, resistance frequency was reduced to 4%. In another packinghouse where Penbotec was used during 2005-2009 but only Scholar was used on 2010 crops, and the frequency of pyrimethanil resistant strains was reduced from 7% in 2010 to 1% in 2011. The results clearly demonstrated the benefit of rotation of postharvest fungicides for drench.

- In other three packinghouses, neither Penbotec nor Scholar had been widely used before 2010. No pyrimethanil resistance was detected in two of the three packinghouses, and 1.8% of the isolates from one packinghouse were resistant to pyrimethanil. The findings support our recommendations on rotation of postharvest fungicides as a drench, and fungicide resistance management practices need to be implemented in the industry.

- The frequency of Pristine-resistant strains in apple orchards where Pristine had been used during 2005-1010 declined from 2010 to 2011 season. Fungicides used in these orchards and perhaps other factors such as competitive disadvantage of Pristine-resistant strains may affect the dynamic of Pristine-resistant populations. The results may suggest that Pristine can still be used and remain effective when the resistant populations decline.

- Reduced rates of tank-mixture of Pristine and Topsin M significantly reduced incidence of gray mold caused by the Pristine-sensitive strain but not the Pristine-resistant strain. In 2011, we repeated the experiment with an emphasis on a tank-mixture of full label rates of Pristine and Topsin for control of Pristine-resistant strains. On 2011 crops, Pristine and Topsin mixture provided better control for Pristine-sensitive strain than Pristine or Topsin alone. For Pristine-resistant isolate, Pristine+Topsin mixture and Topsin alone provided better control than Pristine alone. Pristine+Ziram mixture was more effective than Pristine alone or Ziram alone for control of Pristine-sensitive isolate, but was less effective for control of Pristine-resistant isolate than for control of Pristine-sensitive isolate.

- Boscalid only delayed conidial germination and had no fungicidal activity against *Penicillium expansum*. Pyraclostrobin and Pristine appeared to only have suppressive activity against *P. expansum*.

- Boscalid resistance and pyraclostrobin resistance in *B. cinerea* were stable. However, boscalid-resistant and pyraclostrobin-resistant strains had disadvantages in competing with fungicide-sensitive strains of *B. cinerea*, suggesting that if the use of these fungicides is discontinued in the orchard, frequency of resistant populations will likely decline.

- Although DPA is not a fungicide, TBZ-resistant isolates became sensitive to DPA and a DPA treatment significantly controlled gray mold caused by TBZ-resistant strains. Resistance to the AP fungicides compromised the efficacy of pyrimethanil as a postharvest treatment for control of gray mold. Fludioxonil was effective against all phenotypes. The results suggest that the use of AP fungicides in the orchards should be limited in order to minimize the risk of development of resistance to pyrimethanil.

- Preharvest applications of Serenade MAX or Sonata did not significantly reduce postharvest rots in comparison with the nontreated control.

Methods:

Blue mold-decayed fruit were sampled from grower lots that had been drenched with Penbotec or Scholar from commercial fruit packinghouses. Isolates of *Penicillium* spp. were identified to species. Isolates of *P. expansum* were screened for resistance to fludioxonil, pyrimethanil, and TBZ.

Baseline sensitivities of *P. expansum* to pyraclostrobin, boscalid and Pristine were determined. Non-exposed isolates were used to establish distribution of baseline sensitivity of *P. expansum* to these fungicides.

Frequency of Pristine-resistant isolates of *B. cinerea* in apple orchards was determined. Apple fruit were collected from eight orchards 2-3 weeks before harvest. Isolation of *B. cinerea* from the calyx tissue of the fruit or from the surface of the fruit was attempted. Isolates were then tested for resistance to pyraclostrobin, boscalid and Pristine on fungicide-amended agar media.

Biological characteristics of pyraclostrobin-resistant and boscalid-resistant strains of *B. cinerea*, including resistance stability, fitness parameters (mycelial growth, spore production, virulence on apple fruit, etc.), ability to compete with fungicide-sensitive strains, and cross-resistance to other fungicides, were determined.

An experiment was conducted in a research apple orchard. Topsin, Pristine, and their mixture were applied within one week before harvest, and trees receiving no treatment served as a control. After harvest, fruit were immediately transported into the laboratory. Fruit were puncture-wounded, inoculated with different strains of the pathogen, and stored in storage for decay development.

Sensitivity to DPA, fludioxonil and pyrimethanil in Pristine-resistant isolates of *B. cinerea* was tested. To evaluate postharvest fungicides and DPA for control of Pristine-resistant *B. cinerea* on fruit, apple fruit were wounded and inoculated with Pristine-resistant or Pristine-sensitive isolate. Apples were treated with either sterile water as controls or one of the following chemical solutions: DPA, Scholar, Penbotec, DPA+ Scholar, and DPA+Penbotec. Fruit were stored in RA for decay development.

In a commercial organic Fuji orchard, Serenade MAX (*Bacillus subtilis* strain QST 713) and Sonata (*Bacillus pumilus* strain QST 2808) as preharvest sprays were evaluated for postharvest decay control.

Results & Discussion:

Monitoring resistance of P. expansum to pyrimethanil and fludioxonil

In 2010, 389 *P. expansum* isolates were obtained. Pyrimethanil-resistant strains were detected in two packinghouses where Penbotec (pyrimethanil) had been used annually as a postharvest drench since 2005. Approximately 85% of the *P. expansum* isolates obtained from packinghouse A were resistant to pyrimethanil, and 7% of the isolates from packinghouse B were resistant to pyrimethanil (Table 1). No pyrimethanil-resistant strains were detected in the other three packinghouses where Penbotec was used on 2009 crops but no or little use in the past.

All isolates were sensitive to fludioxonil. Approximately 86% and 11% of the isolates were resistant to TBZ in packinghouses A and B, respectively. TBZ-resistant strains were also present in other packinghouses, indicating that TBZ-resistant strains remained in *P. expansum* populations even after TBZ was not used.

In 2011, 410 *P. expansum* isolates were obtained (Table 1). In both Packinghouse A and B, Penbotec (pyrimethanil) was used as a postharvest drench from 2005 to 2009. On the 2010 crops, in packinghouse A, some lots were drenched with Scholar+DPA and some lots with Penbotec. Over 90% of the isolates were resistant to pyrimethanil, while on the fruit drenched with Scholar in 2010, resistance frequency was reduced to 4%. The packinghouse B switched to Scholar on 2010 crops, and the frequency of pyrimethanil resistant strains was reduced from 7% in 2010 (reported in 2010) to 1% in 2011.

In packinghouse A, all isolates obtained from Penbotec-drenched fruit were resistant to TBZ, whereas 12.5% of the isolates from Scholar-drenched fruit were resistant to TBZ. TBZ-resistant strains were also present in other packinghouses but at a low level.

Some isolates showed reduced sensitivity to fludioxonil. As this was the first time that we found strains with reduced sensitivity to fludioxonil, we are currently re-testing these isolates to confirm whether the reduced sensitivity is stable.

Previously we reported the occurrence of pyrimethanil resistance in *P. expansum* in Packinghouses A and B as a result of annually repeated use of Penbotec as a postharvest drench from 2005 to 2009. Since 2010, packinghouses followed our recommendations on resistance management and started rotation of postharvest fungicides as drench. The data from these two packinghouses clearly indicated that switching to Scholar on 2010 crops significantly reduced the frequency of pyrimethanil resistant strains. In Packinghouses C, D and E, neither Penbotec nor Scholar had been widely used before 2010. No pyrimethanil resistance was detected in these three packinghouses. The findings support our recommendations on rotation of postharvest fungicides as a drench, and fungicide resistance management practices need to be implemented in the industry.

Table 1. Monitoring of pyrimethanil resistance in *Penicillium expansum* from apples in 2010

Source	**Drench Treatment**	**# isolates of *P. expansum***	**# isolates resistant to pyrimethanil**	**# isolates resistant to fludioxonil**	**# isolates resistant to thiabendazole**
Packinghouse A	Penbotec	177	150	0	152
Packinghouse B	Penbotec	129	9	0	14
Packinghouse C	Penbotec	26	0	0	2
Packinghouse D	Penbotec	29	0	0	16
Packinghouse E	Penbotec	28	0	0	1

Table 2. Monitoring of pyrimethanil resistance in *Penicillium expansum* from apples in 2011

Packing house	**Drench Treatment**	**# isolates of *P. expansum***	**# isolates resistant to pyrimethanil**	**# isolates resistant to thiabendazole**
Packinghouse A	Scholar+DPA	48	2	6
Packinghouse A	Penbotec	118	113	115
Packinghouse B	Scholar or Scholar+DPA	99	1	7
Packinghouse C	Penbotec	55	1	2
Packinghouse D	Scholar	31	0	1
Packinghouse E	Penbotec	40	0	0

Control of blue mold incited by pyrimethanil-resistant P. expansum

Postharvest fungicides were evaluated for control pyrimethanil-resistant strains on apple fruit. Penbotec at label rate (16 fl oz/100 gallon water) only partially controlled blue mold incited by a low-resistance strain (Table 3), and failed to control blue mold caused by strains exhibiting moderate or high resistance to pyrimethanil. Scholar was effective to control pyrimethanil-resistant strains regardless of pyrimethanil-resistant phenotypes (Table 3). Because all pyrimethanil-resistant strains also were resistant to TBZ, a postharvest treatment with TBZ did not provide satisfactory control of blue mold incited by strains that were resistant to both TBZ and pyrimethanil.

Table 3. Effectiveness of postharvest fungicides for control of blue mold incited by different phenotypes of pyrimethanil-resistant strains of *Penicillium expansum*.

Isolate	**Phenotype**	**Treatment (%)**	**Decay Incidence %**	**Lesion size (mm)**
8841	$TBZ^RFlu^SPyr^{HR}$	Nontreated	95 a	28.4 a
		Pyrimethanil	93.3 a	26.8 a
		Fludioxonil	0 b	0 b
8818	$TBZ^RFlu^SPyr^{MR}$	Nontreated	98.3 a	32.43a
		Pyrimethanil	92.5 b	26.7 a
		Fludioxonil	0 c	0 b
8873	$TBZ^RFlu^SPyr^{LR}$	Nontreated	95 a	29.2 a
		Pyrimethanil	68 b	24.6 a
		Fludioxonil	0 c	0 b
8391	$TBZ^RFlu^SPyr^S$	Nontreated	98 a	31.1 a
		Pyrimethanil	0 b	0 b
		Fludioxonil	0 b	0 b
8692	$TBZ^SFlu^SPyr^S$	Nontreated	93 a	31.1 a
		Pyrimethanil	0 b	0 b
		Fludioxonil	0 b	0 b

TBZ=thiabendazole, Flu=fludioxonil, Pyr=pyrimethanil, R=resistant, S=sensitive, HR=high resistance, MR=moderate resistance, LR=low resistance

Monitoring Pristine resistance in B. cinerea in apple orchards

We monitored Pristine resistance in *B. cinerea* in five apple orchards. Pristine had been used for 5-6 years in these orchards. Except in orchard D, the frequency of Pristine-resistant strains in these orchards declined from 2010 to 2011 season. Fungicides used in these orchards and perhaps other factors such as competitive disadvantage of Pristine-resistant strains may affect the dynamic of Pristine-resistant populations. The data we reported in the past year indicated that Pristine-resistant strains cannot compete well with Pristine-sensitive strains on apple fruit. The results may suggest that Pristine can still be used and remain effective when the resistant populations decline.

Table 4. Frequency of Pristine-resistant *B. cinerea* in 2011 from commercial Gala orchards where Pristine had been used

Orchard	Number of isolates	Frequency of Pristine-resistant isolates (%)	
		2010 season	2011 season
A	50	45.9	6.0
B	24	54.1	37.5
C	25	52.3	20.0
D	35	17.2	17.1
E	35	13.3	5.7

Biological characteristics of Pristine-resistant strains of B. cinerea

Resistance stability and competitive ability of pyraclostrobin resistance and boscalid resistance in *B. cinerea* were studied. The results have been presented in the 2010 progress report. In summary, our results indicated that boscalid resistance and pyraclostrobin resistance in *B. cinerea* were stable. However, boscalid-resistant and pyraclostrobin-resistant strains had disadvantages in competing with fungicide-sensitive strains of *B. cinerea*, suggest that if the use of these fungicides is discontinued in the orchard, frequency of resistant populations will likely decline.

Control of gray mold caused by Pristine-resistant Botrytis cinerea

Field experiments were conducted on Fuji crops in 2010 and 2011. Fungicide treatments were applied one week or two weeks (for ziram-containing treatments) before harvest. The fruit were inoculated with Pristine-sensitive or Pristine-resistant strains of *B. cinerea*.

On 2010 crops, Pristine at 14.5 oz/A and the new fungicide BAS 703 at 4.11 fl oz/A significantly reduced incidence of gray mold caused by the Pristine-sensitive strain but not the Pristine-resistant strain. Reduced rates of tank-mixture of Pristine and Topsin M significantly reduced incidence of gray mold caused by the Pristine-sensitive strain but not the Pristine-resistant strain. Reduced rates of tank-mixture of BAS 703 and Topsin M significantly reduced incidence of gray mold caused by either pristine-sensitive or –resistant strain but was more effective against Pristine-sensitive strain.

On 2011 crops, Pristine and Topsin mixture provided better control for Pristine-sensitive strain than Pristine or Topsin alone (Table 6). For Pristine-resistant isolate, Pristine+Topsin mixture and Topsin alone provided better control than Pristine alone. Pristine+Ziram mixture was more effective than Pristine alone or Ziram alone for control of Pristine-sensitive isolate, but was less effective for control of Pristine-resistant isolate than for control of Pristine-sensitive isolate. New fungicide Merivon (not yet registered) also was effective for control of gray mold.

Table 5. Efficacy of preharvest fungicide programs for control of Pristine-resistant strains of *Botrytis cinerea* on apple fruit in 2010-2011

Treatment	Incidence of gray mold (%)	
	Pristine-sensitive strain	Pristine-resistant strain
Control: No Fungicide	100.0 a	98.8 a
Pristine 14.5 oz + Sylgard	41.3 cd	100.0 a
BAS 70301F 4.11 fl oz + Sylgard	58.3 bc	95.0 ab
Topsin 1 lb + Sylgard	68.8 b	72.5 c
Pristine 10.9 fl oz + Topsin .75 lb + Sylgard	32.5 d	98.8 a
BAS 70301F 3.08 + Topsin .75 lb+Sylgard	52.5 bc	81.3 bc

Values with the same letter in the same column are not significantly different based on the Waller-Duncan test (P = 0.05).

Table 6. Efficacy of preharvest fungicide programs for control of Pristine-resistant strains of *Botrytis cinerea* on apple fruit in 2011-2012

Treatment	Incidence of gray mold (%)	
	Pristine-sensitive strain	Pristine-resistant strain
Control: No fungicide	100 a	100 a
Ziram 6 lb	75 b	61.25 ef
Merivon 4.0 oz + Ziram	23.75 e	52.5 f
Pristine 14.5 oz + Ziram	13.75 e	72.5 def
Merivon 4.0 oz	68.75 bc	88.75 bcd
Pristine 14.5 oz	56.25 cd	97.5 ab
Topsin 16 oz	66.25 bc	77.5 de
Merivon 4.0 oz+Topsin	48.75 d	61.25 ef
Pristine+Topsin	23.75 e	83.75 cd
Merivon 5.5 oz	58.75 cd	91.25 bc

Values with the same letter in the same column are not significantly different based on the Waller-Duncan test (P = 0.05). Merivon has not yet been registered for use on apple.

Sensitivity of P. expansum to Pristine

At 1 µg/ml of pyraclostrobin, no conidial germination was observed within 30 h of incubation at 20°C. Germination was completely inhibited at 2,000 µg/ml of pyraclostrobin for up to 7 days, but conidia were able to germinate when they were transferred to plain PDA. All of the isolates did not germinate at 5 µg/ml boscalid after 20 h of incubation at 20°C, but conidia were swollen. At 30 h of incubation, conidia were able to germinate at 100 µg/ml boscalid, indicating that boscalid only delayed conidial germination. The range of EC50 values of Pristine was from 0.009 to 0.019 µg/ml, with a mean of 0.013 µg/ml (Fig. 1). Our results indicated that boscalid only delayed conidial germination and had no fungicidal activity against *P. expansum*. Pyraclostrobin and Pristine appeared to only have suppressive activity against *P. expansum*.

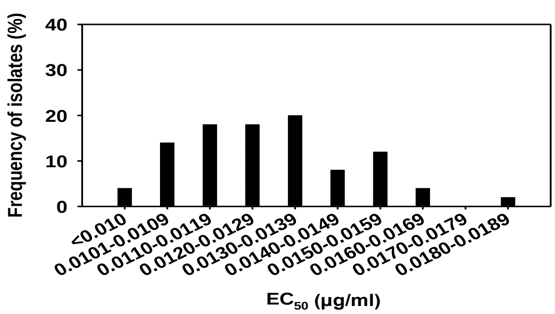

Fig. 1. Distribution of sensitivity of *Penicillium expansum* to Pristine.

Sensitivity to DPA and control of Pristine-resistant strains of B. cinerea with postharvest fungicides and DPA

Sensitivity to DPA, TBZ, fludioxonil and pyrimethanil in Pristine-resistant isolates of *B. cinerea* was tested. All Pristine-resistant isolates that were sensitive to TBZ were insensitive to DPA. However, Pristine-resistant isolates that were also resistant to TBZ became sensitive to DPA. All isolates remained sensitive to fludioxonil but some were resistant to pyrimethanil, likely because cyprodinil (Vangard) had been used in some of these orchards. The results indicated that Pristine resistance does not change the sensitivity of the isolates to DPA and that DPA may be able to control TBZ-resistant strains of *B. cinerea*.

An experiment was conducted during 2010-2011 storage season to evaluate postharvest fungicides and DPA alone or their combinations for control of gray mold caused by Pristine-resistant and/or MBC-resistant strains of *B. cinerea*. Although DPA is not a fungicide, MBC-resistant isolates became sensitive to DPA and a DPA treatment significantly controlled gray mold caused by TBZ-resistant strains (Table 7). Resistance to the AP fungicides compromised the efficacy of pyrimethanil as a postharvest treatment for control of gray mold. Fludioxonil was effective against all phenotypes.

Table 7. Effectiveness of DPA with or without postharvest fungicides for control of gray mold incited by various fungicide-resistant phenotypes of *Botrytis cinerea*

		Incidence (%)	
Phenotype[x]	Treatment	DPA -	DPA +
$MBC^RAP^RQoI^RSDHI^R$	Control	100 aA[y]	9.4 bB
	TBZ	100 aA	16.3 aB
	Fludioxonil	0 c	0 c
	Pyrimethanil	48.8 b	19.4 a
$MBC^RAP^SQoI^RSDHI^R$	Control	100 aA	13.8 aB
	TBZ	100 aA	16.3 bB
	Fludioxonil	0 b	0 c
	Pyrimethanil	0 b	0 c
$MBC^RAP^SQoI^SSDHI^S$	Control	100 aA	20.6 aB
	TBZ	100 aA	11.9 bB
	Fludioxonil	0 b	0 c
	Pyrimethanil	0 b	0 c
$MBC^SAP^SQoI^RSDHI^S$	Control	100 a	100 a
	TBZ	0 bB	5 bA
	Fludioxonil	0 b	0 c
	Pyrimethanil	0 b	0 c
$MBC^SAP^SQoI^SSDHI^R$	Control	100 a	100 a
	TBZ	2.5 bB	26.3 bA
	Fludioxonil	0 c	5.6 c
	Pyrimethanil	0 c	3.1 c
$MBC^SAP^SQoI^SSDHI^S$	Control	98.1 a	100 a
	TBZ	0 bB	6.9 bA
	Fludioxonil	0 b	0 c
	Pyrimethanil	0 b	0 c

[x] MBC = TBZ, thiophanate-methyl; AP = cyprodinil, pyrimethanil; QoI = pyraclostrobin; SDHI = boscalid.
[y] Values are the means of pooled data from the two runs of the experiment. Values followed by the same lowercase letter within a column in each isolate are not significantly different according to the ANOVA and LSD at $P = 0.05$. Values followed by the same capital letter within a row are not significantly different according to t-test at $P = 0.05$. Data were arcsine-transformed before analysis.

Preharvest biocontrol agents for control of postharvest fruit rots

Experiments were conducted in an organic Fuji orchard near Quincy in both 2010-11 and 2011-12 seasons. Biocontrol agents Sonata and Serenade were applied to the fruit 10 days and 1 day before harvest. Fruit were harvested and wounded with a finish-nail head to simulate puncture wounds. Natural inoculum was used in this study. Preharvest applications of Serenade MAX or Sonata did not significantly reduce postharvest rots in comparison with the nontreated control (Table 8).

Table 8. Efficacy of preharvest applications of Serenade and Sonata for control of postharvest fruits rots on organic Fuji apples in 2010-11 and 2011-12 seasons

Treatment	**Rots (%) in 2010-2011 season**	**Rots (%) in 2011-2012 season**
Nontreated	11.04 a	4.48 a
Serenade MAX at 10 and 1 day before harvest	6.88 a	4.38 a
Sonata at 10 and 1 day before harvest	6.98 a	4.17 a

Executive Summary

This report is a summary of a two-year project conducted in 2010 and 2011. Part of the research was completed in 2012 because of the postharvest nature of the project. The objectives of the project were to monitor, characterize, and manage fungicide resistance in *Penicillium expansum* and *Botrytis cinerea*, two major postharvest pathogens of apples. The goal was to develop sustainable postharvest decay control programs.

Blue mold caused by *P. expansum* and gray mold caused by *B. cinerea* are major postharvest diseases of apples. In the present project, we monitored pyrimethanil resistance and fludioxonil resistance in *P. expansum*. Resistance to pyrimethanil (Penbotec) has developed in *P. expansum* populations in some packinghouses where the fungicide as a postharvest drench has been used annually for 4-5 consecutive years. In one packinghouse, over 90% of the isolates were resistant to pyrimethanil when Penbotec (pyrimethanil) was again used on 2010 crops, while on the fruit drenched with Scholar in 2010, resistance frequency was reduced to 4%. In another packinghouse where Penbotec was used during 2005-2009 but only Scholar was used on 2010 crops, the frequency of pyrimethanil resistant strains was reduced from 7% in 2010 to 1% in 2011. In the other three packinghouses, neither Penbotec nor Scholar had been widely used before 2010. No pyrimethanil resistance was detected in two of the three packinghouses, and 1.8% of the isolates from one packinghouse were resistant to pyrimethanil. The findings support our recommendations on rotation of postharvest fungicides as a drench, and fungicide resistance management practices need to be implemented in the industry.

Boscalid resistance and pyraclostrobin resistance in *B. cinerea* were stable. However, boscalid-resistant and pyraclostrobin-resistant strains had disadvantages in competing with fungicide-sensitive strains of *B. cinerea*, suggesting that if the use of these fungicides is discontinued in the orchard, frequency of resistant populations will likely decline. The frequency of Pristine-resistant strains of *B. cinerea* in apple orchards where Pristine had been used during 2005-1010 declined from 2010 to 2011 season. Fungicides used in these orchards and perhaps other factors such as competitive disadvantage of Pristine-resistant strains may affect the dynamic of Pristine-resistant populations. The results may suggest that Pristine can still be used and remain effective when the resistant populations decline.

Pristine and Topsin mixture provided better control for Pristine-sensitive strain than Pristine or Topsin alone. For Pristine-resistant isolate, Pristine+Topsin mixture and Topsin alone provided better control than Pristine alone. Pristine+Ziram mixture was more effective than Pristine alone or Ziram alone for control of Pristine-sensitive isolate, but was less effective for control of Pristine-resistant isolate than for control of Pristine-sensitive isolate.

Boscalid only delayed conidial germination and had no fungicidal activity against *P. expansum*. Pyraclostrobin and Pristine appeared to only have suppressive activity against *P. expansum*.

All Pristine-resistant isolates that were sensitive to TBZ were insensitive to DPA. However, Pristine-resistant isolates that were also resistant to TBZ became sensitive to DPA. The results indicated that Pristine resistance does not alter the sensitivity of the isolates to DPA but there is a negative cross resistance between TBZ and DPA. Although DPA is not a fungicide, TBZ-resistant isolates became sensitive to DPA and a DPA treatment significantly controlled gray mold caused by TBZ-resistant strains. Resistance to the AP fungicides compromised the efficacy of pyrimethanil as a postharvest treatment for control of gray mold. Fludioxonil was effective against all phenotypes. The results suggest that the use of AP fungicides in the orchards should be limited in order to minimize the risk of development of resistance to pyrimethanil.

Preharvest applications of Serenade MAX or Sonata did not significantly reduce postharvest rots in comparison with the nontreated control.