FINAL REPORT

Project Title: Controlling postharvest disorders of pears during storage and export

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Total Project F	Request: Year 1	: \$25,090	Year 2: \$25,751	Year 3: \$26,431		

Other funding sources: None

Budget

Organization Name: Agricultural Research FoundationContract Administrator: Russ KarowTelephone: 541-737-4066Email address: Russell.Karow@oregonstate.edu

Item	2013	2014	2015
Salaries	13,0881	13,481	13,885
Benefits	$1,250^2$	1,300	1,352
Wages	6,715 ³	6,917	7,124
Benefits	5374	553	570
Equipment			
Supplies	3,000 ⁵	3,000	3,000
Travel	5006	500	500
Miscellaneous			
Total	25,090	25,751	26,431

Footnotes:

¹Postdoctoral Research Associate (Dr. Xingbin Xie): 1/3 FTE. 3% increase is factored into Year 2 and 3.

²OPE: 1/3 FTE. 4% increase is factored into Year 2 and 3.

³Wages: 500hr for a Biological Science Tech. at \$13.43/hr. 3% increase is factored into Year 2 and 3.

⁴OPE: 8% of the wage.

⁵Supplies: maintaining cold rooms, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals.

⁶Travel: field trips to packinghouses and orchards.

OBJECTIVES

- 1. Control senescence disorders and extend storability of summer pears by ethylene inhibitors
- 2. Reduce pear scuffing by wax coatings
- 3. Evaluate the efficacy of a premix formulation of Difenoconazole + Fludioxonil on storage decays

SIGNIFICANT FINDINGS:

Objective 1. Control senescence disorders and extend storability of summer pears by ethylene inhibitors

1. Pre-harvest AVG efficacy on extending storability of 'Bartlett' is affected by application rate, timing, and fruit harvest maturity.

- AVG at 60-120ppm applied 1 week before harvest (WBH) extended storability of 'Bartlett' for H1 (H1=19lb) and H2 (12d after H1, H2=18lb) fruit. H3 (17d after H1, H3=17lb) fruit did not response to the AVG treatments.
- AVG applied 2 WBH1 had little effect on storability.
- AVG applied 1 WBH1 did not affect the initial harvest maturity (H1), but delayed fruit maturation on the tree about 5d for H2 and H3 fruit.

2. Postharvest 1-MCP efficacy on extending storability of 'Bartlett' is inconsistent at commercial application among lots and years. To ensure a consistent 1-MCP efficacy:

- Harvest fruit at \geq 19lb, especially for fruit from higher production elevations (i.e., >1,000ft).
- Treat fruit within 10-12 days after harvest. Eliminate field heat quickly and store fruit at 30°F during the treatment delay.
- Vent out exogenous ethylene (if > 300 ppb) in the treating room before 1-MCP treatment.

3. Pre-harvest AVG or postharvest 1-MCP treatments extend storage life of 'Starkrimson'.

- 'Starkrimson' produces a higher amount of ethylene and has a higher respiration rate and therefore a shorter storage life compared to other PNW pear cultivars.
- AVG at 60-120ppm applied 1 week before harvest extends 'Starkrimson' storage life without significant effect on ripening capacity following cold storage.
- 1-MCP at 300ppb extends 'Starkrimson' storage life to 4 months at 30°F. However, it took 2 weeks to ripen at 68°F following 4 months of cold storage.

Objective 2. Reduce pear scuffing by wax coatings

- 'Comice', 'Anjou', 'Bartlett', 'Bosc', and 'Starkrimson' are in the increasing order regarding susceptibility to anaerobic injury.
- Compared to the commercial standard rate of 5-6% solid, Carnauba wax coating at solid of 7-8% decreases friction forces and therefore reduce scuffing without causing anaerobic flavor of 'Comice' pear. Sugar-ester edible coating at 0.5-1.0% a.i. reduces scuffing and maintained green color without affecting ripening capacity and flavor of 'Bartlett' pear.
- Ethoxyquin at 1000ppm mixed in wax coating may slow down chlorophyll degradation and reduce scuffing expression of 'Comice'.

Objective 3. Evaluate the efficacy of a premix formulation of Difenoconazole + Fludioxonil

• The pre-mix formulation of Difenoconazole + Fludioxonil (Syngenta product) applied as drenching at 16 oz. per 100 gallons controls blue and gray mold decays at levels equivalent to Penbotech or Scholar alone.

• The different modes of action between Difenoconazole and Fludioxonil in the pre-mix may retard resistance development in the pathogens.

METHODS

Objective 1. Extend storability of summer pears by ethylene inhibitors

'Bartlett'. The effects of harvest maturity (19-16lb), delayed treatment (1-12d), production elevations (500-2,000ft), and exogenous ethylene (0-1ppm) on efficacy of 1-MCP were studied. A 40m³ cold room at 32°F was used to treat fruit with 1-MCP at the commercial rate of 300ppb for 24h. Treated fruit were stored at 30°F. Fruit color, I_{AD}, IEC, FF, senescent scald, internal breakdown, and flavor were evaluated before and during storage for 4 months. The effects of pre-harvest AVG spray rate (30, 60, and 120ppm) and timing [1 and 2 WBH1 (weeks before H1)] on storability of 'Bartlett' fruit at three harvest maturities [H1: when control fruit firmness (CFF) \approx 19lb; H2: 12 d after H1 when CFF \approx 18lb; and H3: 17 d after H1 when CFF \approx 17lb] were measured with respect to ethylene production, storage quality and ripening capacity during 5 months of storage at 30°F. **'Starkrimson'.** AVG at 30, 60, and 120 ppm was sprayed 1 week before commercial harvest. Fruit quality and ripening capacity were determined after 1, 2, 3, and 4 months of cold storage at 30°F. Postharvest treatment with 1-MCP at 300 ppb was as the same as described for 'Bartlett'.

Objective 2. Reducing pear scuffing by wax coatings and/or ethoxywuin

Effects of carnauba wax solids (0, 5%, 10%, 15%, and 20%) and sugar-ester edible coating a.i. (0.1%, 0.5%, and 1.0%) on fruit respiration and ripening physiology, anaerobic physiology, fruit quality, and scuffing of the major PNW cultivars ('Starkrimson', 'Bartlett', 'Bosc', 'Comice', and 'd'Anjou') were studied. The effect of ethoxyquin mixed in wax on scuffing was studied on 'Comice' pear

Objective 3. Evaluate the efficacy of a premix formulation of Difenoconazole + Fludioxonil on storage decay of pears

A pre-mix formulation of Fludioxonil + Difenoconazole, Difenoconazole alone, Scholar, and Penbotec were obtained from Syngenta. Artificially inoculated 'Bosc' pear fruits with spore solutions of *Botrytis cinerea* and *Penecillium expansum* were drenched with the fungicides at label recommended rates. Decay incidence, decay severity, and sporulation were evaluated after 3-5 months of storage at 30°F.

RESULTS

1. Ensure a consistent 1-MCP efficacy on extending storability of 'Bartlett' pears

1-MCP efficacy on inhibiting senescence of 'Bartlett' pears has been reported being inconsistent from year to year and from lot to lot in the PNW. The effects of harvest maturity, orchard elevations, delayed treatment after harvest, holding temperature during treatment delay, and exogenous ethylene concentration in treating room on 1-MCP efficacy were studied in 2012 and 2013.

1.1. Effect of 'Bartlett' harvest maturity on 1-MCP efficacy.

There were 3 harvest maturities: H1=19lb, H2=17.2lb, H3=16.5lb. 1-MCP treatment maintained fruit peel chlorophyll and FF for H1 and H2 fruit without senescent disorders (yellowing, senescent scald and internal breakdown) for 4-5 months of cold storage. However, 1-MCP treated F3 fruit lost chlorophyll and FF significantly after 2 months and developed senescent disorders after 4 months of cold storage at 30°F. Ripening capacity after storage was retarded especially for H1 and H2 (Fig. 1).



Fig. 1. Effect of harvest maturity on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' pears following cold storage at 30°F.

After 3 months of cold storage, fruit were transferred to a cold room at 41°F for 3 weeks to simulate export transit conditions. 1-MCP treatment maintained higher peel chlorophyll content and FF for only H1 fruit after the simulated export transit. Both H2 and H3 fruit treated with 1-MCP lost chlorophyll and FF significantly and developed senescent scald and IB after the simulated transit (Fig. 2).



Fig. 2. Effect of harvest maturity on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' pears following 3 months of cold storage at 30 °F plus 3 weeks at 41 °F.

1.2. Effect of delayed treatment after harvest on 1-MCP efficacy.

It may take 10-12d or longer time to fill a storage room. 'Bartlett' fruit were harvested at 19-17lbs. Fruit were stored at cold rooms at 30, 37, and 41°F for 12d until 1-MCP treatment. IEC did not increase at 30°F, but increased at 37 and 41 °F during the 12d delay. A delayed treatment of fruit that have been stored at 30 °F for 12d did not reduce 1-MCP efficacy on maintaining fruit peel chlorophyll and FF of 'Bartlett' pears after 4 months of cold storage, compared to treating fruit immediately after harvest. However, storing fruit at 37 and 41 °F for 12d after harvest reduced 1-MCP efficacy on maintaining quality of 'Bartlett' after 4 months of cold storage (Fig. 3).



Fig. 3. Effect of treatment delays on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' after 4 months of cold storage at 30 °F.

1.3. Effect of production elevation on 1-MCP efficacy.

'Bartlett' fruit were harvested at 3 maturities based on FF from two elevations: 500ft and 2000ft. at the same FF, fruit from the higher elevation (2000ft) had higher IEC, especially for H2 (17lbf) and H3 (16.5lbs). H2 and H3 from elevation of 2000ft reduced 1-MCP efficacy on maintaining color and FF after 4 months of cold storage, compared to H1.

1.4. Effect of exogenous ethylene in treating room on 1-MCP efficacy.

Exogenous ethylene at 300ppb in treating room reduced 1-MCP efficacy on maintaining chlorophyll and FF and senescent scald-free after 4 months of cold storage (Fig. 4).



Fig. 4. Effect of exogenous ethylene in treating room on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' following 4 months of cold storage at 30°F.

2. Pre-harvest ReTain[®] spray efficacy on extending storability of 'Bartlett' pears is affected by application rate, timing, and fruit harvest maturity

2.1. Ethylene production

In H1, the control fruit started to produce significant amount of ethylene after 2 months of cold storage (Fig. 5A). AVG at 60 and 120ppm applied 1 WBH1 reduced ethylene production rate significantly during 2-5 months of storage. Compared to 60ppm, AVG at 120ppm 1 WBH1 further reduced ethylene production rate numerically but not at statistically significant level (p = 0.05) during the experimental period. In contrast, AVG at 30ppm applied 1 WBH1 and AVG at 120ppm applied 2 WBH1 did not inhibit ethylene production compared to the control. For H2 fruit following 4 months of storage, similar to H1 fruit, AVG at 60 and 120ppm applied 1 WBH1 reduced ethylene production, but AVG at 30 mg L⁻¹ applied 1 WBH1 and 120 mg L⁻¹ applied 2 WBH1 did not affect ethylene production in H3 fruit following 4 months of storage was not affected by the AVG treatments (Fig. 5B).



Fig. 5. Effects of pre-harvest AVG sprays on ethylene production of 'Bartlett' pears with three harvest maturities (H1, H2, and H3) on day 1 at 68°F following cold storage at 30°F for 1-5 months in H1 and 4 months in H2 and H3.

2.2. Fruit storage quality

In H1 fruit, FF, I_{AD}, and TA decreased gradually in all the treatments. Their losses were not affected by AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1, but slowed down significantly by AVG at 60 and 120ppm applied 1 WBH1 during 5 months of storage (Fig. 6). In H2 fruit following 4 months of storage, AVG at 60 and 120ppm applied 1 WBH1 maintained higher FF, I_{AD}, and TA, but AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1 did not affect the losses of FF, I_{AD}, and TA. H3 fruit did not response to the AVG treatments in terms of the losses of FF, I_{AD}, and TA (data not shown). SSC increased in a small magnitude in each of the three maturities during storage, but it was not affected by the AVG treatments.



Fig. 6. Effects of pre-harvest AVG sprays on fruit flesh firmness and peel chlorophyll content (I_{AD}) of 'Bartlett' pears on day 1 at 68°F following cold storage at 30°F for 1-5 months in H1.

2.3. Senescence disorders

In the control, H1 and H2 fruit developed senescence disorders of 30.3% and 35.0% after 5 and 4 months of storage, respectively (Fig. 7). AVG at 60ppm applied 1 WBH1 reduced senescence disorders to 5.6% and 16.1% in H1 and H2 fruit after storing for 5 and 4 months, respectively. Senescence disorders in H1 and H2 fruit were not affected by AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1. Compared to AVG at 60ppm, AVG at 120ppm did not improve its efficacy on reducing senescence disorders. H3 control fruit developed 49.5% senescence disorders following 4 months of storage and the AVG treatments did not affect the senescence disorders significantly.



Fig. 7. Effects of pre-harvest AVG sprays on senescence disorders of 'Bartlett' pears with three harvest maturities (H1, H2, and H3) on day 7 at 68°F following cold storage at 30°F.

2.4. Ripening capacity

In H1, the control fruit developed ripening capacity following 1-4 months, but developed mealy texture with increased EJ > 650 mL kg⁻¹ within 7d at 68°F following 5 months of cold storage (Fig. 8A&C). Following 1 month of cold storage, fruit treated with AVG at 60ppm applied 1 WBH1 could ripen to FF = 5.5lb with EJ = 649 mL kg⁻¹ in 7 d at 68°F, however, fruit treated with AVG at 120ppm applied 1 WBH1 did not develop ripening capacity. Fruit treated with AVG at 60 and 120ppm applied 1 WBH1 developed ripening capacity with FF < 5lb and EJ < 650 mL kg⁻¹ after 7 d at 68°F following 2-5 months of cold storage. Compared to the control, the ripening capacity was not affected by AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1. Following 4 months of storage, while H2 fruit in all the treatments developed ripening capacity, the fruit treated with AVG at 60 and 120ppm applied 1 WBH1 had less EJ after 7d at 68°F compared to the control and fruit treated with AVG at 30 mg L⁻¹ applied 1 WBH1 and 120 mg L⁻¹ applied 2 WBH1 (Fig. 8D). In contrast, H3 fruit, regardless of the control and AVG treatments, developed mealy texture with EJ > 650 mL kg⁻¹ after 7d at 68°F following 4 months of cold storage (Fig. 8D).



Fig. 8. Effects of pre-harvest AVG sprays on fruit ripening capacity expressed as flesh firmness and extractable juice on day 7 at 68°F following cold storage at 30°F for 1-5 months in H1 and 4 months in H2 and H3.

3. Extend storability of 'Starkrimson' by preharvest ReTain® or postharvest 1-MCP

3.1. Ethylene production and respiration rate

Control fruit started accumulating IEC at about 0.6ppm after 4 weeks. Thereafter, IEC increased gradually and reached the highest amount of 4.8ppm at 16 weeks of storage at 30°F (Fig. 9). Fruit treated with AVG at 30, 60 and 120ppm started accumulating IEC at 1.7, 0.9, and 0.6ppm, respectively, after 8 weeks and IEC peaked at 4.7, 3.3, and 3.2ppm, respectively, after 16 weeks of storage. There was no difference between AVG at 60 and 120ppm on IEC accumulation. Compared to the AVG treatment, 1-MCP was more effective in inhibiting the IEC during storage. 1-MCP treated fruit started accumulating IEC at 0.1ppm after 8 weeks and had IEC lower than 1.0ppm for the 16 weeks of storage. Ethylene production rate (EPR) in control fruit increased significantly after 4 weeks, increased thereafter and reached a maximum value after 16 weeks. Fruit EPR was not affected by AVG at 30ppm but decreased significantly by AVG at 60 and 120ppm during 4-16 weeks. 1-MCP prevented EPR during 16 weeks of storage and was generally higher than that of AVG at 60 and 120ppm. AVG at 30ppm did not affect RR compared to control. 1-MCP treated fruit maintained the lowest RR which decreased in the first 4 weeks and then increased during 4–16 weeks of storage (Fig. 9).



Fig. 9. Effects of AVG and 1-MCP on internal ethylene concentration (IEC), ethylene production rate (EPR) and respiration rate (RR) of 'Starkrimson' pears during 16 weeks of storage at 30°F.

3.2. Fruit storage quality

AVG sprayed one week before harvest at 60, but not 30 and 120ppm slowed down the FF reduction of fruit on the trees compared to control. At nearly the commercial harvest date, FF of fruit treated with AVG at 0, 30, 60, and 120ppm were 58.7, 58.0, 62.6, and 59.9 N, respectively (Fig. 10). Control fruit decreased FF from 58.7 to 53.1 N and maintained SSC at about 11.5% for 12 weeks of storage at -1.1 °C (Fig. 10). AVG and 1-MCP applications did not affect FF and SSC. TA decreased gradually and lost 40% in control fruit after 16 weeks of storage (Fig. 10). AVG and 1-MCP treatments inhibited TA reduction (Fig. 10). For example, AVG at 30, 60, and 120 μ L L⁻¹ and 1-MCP reduced TA loss from 40% to 28, 20, 28, and 23%, respectively, after 16 weeks of storage.



Fig. 10. Effects of AVG and 1-MCP on FF, SSC, and TA of 'Starkrimson' pears during 16 weeks of storage at 30°F.

3.3. Senescence disorders

After 16 weeks of cold storage, control fruit developed internal breakdown (IB) and decay at 12.3 and 7.1%, respectively. AVG at 30, 60, and 120 μ L L⁻¹ and 1-MCP at 0.3 μ L L⁻¹ reduced IB to 10.5, 2.5, 3.3, and 0%, and decay to 6.6, 1.2, 3.3, and 1.1%, respectively (Fig. 11).



Fig. 11. Effects of AVG and 1-MCP on internal breakdown (IB) and decay of 'Starkrimson' pears after 16 weeks at 30°F.

3.4. Ripening capacity

The control fruit could not ripen immediately after harvest, but developed ripening capacity within 5d at 68°F after 2 weeks of storage at 30°F. Ripening capacity was not affected by AVG at 30 and 60ppm. Fruit treated with AVG at 120ppm developed ripening capacity after 4 weeks of cold storage. Both control and fruit treated with AVG at 30ppm maintained low EJ (i.e., < 600 mL kg⁻¹ FW) and high eating quality (i.e., > 7) between 2–8 weeks of storage and increased EJ and lost eating quality thereafter. Fruit treated with AVG at 60 and 120ppm maintained low EJ and high eating quality between 2–16 and 4–16 weeks of cold storage, respectively. 1-MCP treated fruit could not develop ripening capacity within 5d at 68°F for 16 weeks of storage, but could ripen in 15d at 68°F with high eating quality following 4–16 weeks of cold storage (Fig. 12).



Fig. 12. Effects of AVG and 1-MCP on FF, extractable juice (EJ), and eating quality of 'Starkrimson' pears after 5 d at 68 °F following 16 weeks of storage at 30°F.

4. Reducing pear scuffing.

Pears are more sensitive to high CO₂injury. Therefore, waxes (e.g., carnauba) for pears are lower in solids (5-6%) than those for apples (18-22%). However, low solid waxes are less effective in minimizing scuffing. Fruit were waxed after harvest using a commercial carnauba wax coating at solids of 0, 5, 10, 15, and 20%. After satisfying chill requirement plus 7d at 68°F, 'Starkrimson' and 'Bosc' developed anaerobic metabolism at wax solid of 20%; 'Starkrimson', 'Bosc', 'Bartlett', and 'd'Anjou' developed IB or abnormal ripening at wax solids higher than 10%. Injury was not found in 'Comice' at any of the wax solid treatments (Fig. 13).



Fig. 13. Internal O_2 and CO_2 concentrations affected by wax solids of a commercial carnauba wax coating after fruit ripening of 5 European pear cultivars.

Compared to the commercial wax solids of 5-6%, wax solids at 7-8% plus ethoxyquin at 1000ppm reduced scuffing without negative effect on fruit quality of 'Comice' pear. Carnauba wax coating at higher solids may reduce abrasion force on fruit peel during online processing and ethoxyquin may reduce the enzymatic reaction and therefore expression of the discoloration. Semperfresh at 0.5-1% a.i. reduced scuffing and maintained green color without affecting ripening capacity of 'Bartlett' after long-term storage (Fig. 14).



Fig. 14. Effect of Semperfresh at 0-1.0% a.i. on 'Bartlett' pear appearance after 6 months storage at 30°F.

5. The efficacy of a premix formulation of Difenoconazole + Fludioxonil on storage decay

The pre-mix of Fludioxonil + Difenoconazole, applied as drenching within 18h after inoculation, was very efficient and comparable with Penbotec and Scholar on controlling both blue and gray molds of pears during cold storage (Fig. 15). The rate at 16 oz was more efficient than 11.4 oz on gray mold. Both rates were equal and efficient on blue mold.



Fig. 15. Blue mold and gray mold decays in inoculated 'Bosc' pears treated by experimental and standard fungicides and evaluated after 3 and 5 months of cold storage at 30°F. Rates are in fluid ounces per 100 gallons of water.

EXCUTIVE SUMMARY

Project title: Deliver 1-MCP treated 'd'Anjou' pears with predictable ripening capacity

'Bartlett' and 'Starkrimson' are summer pears with relatively short storage life. Significant losses may occur after long-term cold storage or long-distance shipping due to senescence disorders. For 'Bartlett', a recent trend toward greater fresh market utilization has increased the need for extending 'Bartlett' storage life to prolong the packing and marketing season. For 'Starkrimson', increased export demand has resulted in new challenges for maintaining quality during long-distance transport. The senescence disorders and relatively short storage life of summer pears are the result of increased ethylene production induced by cold storage. Ethylene inhibitors AVG and 1-MCP have the potential to extend storability and reduce senescence disorders of 'Bartlett' and 'Starkrimson'.

1-MCP efficacy on extending storability of 'Bartlett' is inconsistent at commercial application in PNW among production lots and years. This research indicated that to ensure a consistent 1-MCP efficacy, (1) harvest fruit at \geq 19lb, especially for fruit from higher production elevations (i.e., >1,000ft); (2) treat fruit with 1-MCP within 10-12 days after harvest and eliminate field heat quickly after harvest and store fruit at 30°F during the treatment delay; (3) vent out exogenous ethylene (if > 300ppb) in the fumigation room before the 1-MCP treatment. Pre-harvest ReTain[®] spray efficacy on improving storability of 'Bartlett' pears is affected by application rate, timing, and fruit harvest maturity. To maximize AVG efficacy, (1) apply AVG at 60-120ppm at 1 week before harvest 1 (WBH1); (1) harvest fruit at H1 (H1=19lb) and H2 (12d after H1, H2=18lb), H3 (17d after H1, H3=17) fruit did not response to the AVG treatments; (3) AVG applied 2 WBH1 had little effect on any of the storage responses measured; (4) AVG applied 1 WBH1 doesn't delay H1 but extend harvest window for 5d.

'Starkrimson' produces a significant amount of ethylene and has a higher respiration rate and therefore a shorter storage life compared to other PNW pear cultivars. The present study indicated that pre-harvest ReTain® or postharvest 1-MCP treatments extend storage life of 'Starkrimson'. To ensure efficacy, (1) apply AVG at 60-120ppm at 1 week before harvest and harvest fruit at 15-14lb; (2) 1-MCP at 300ppb extend 'Starkrimson' storage life to 4 months at 30°F, however, it takes 2 weeks to ripen at 68°F following 4 months of cold storage.

Sugar-ester edible coating (i.e., Semperfresh) at 0.5-1.0% a.i. or carnauba wax coating at solid of 7-8% decrease friction forces and therefore reduce scuffing without negative effects on ripening and flavor of 'Bartlett' and 'Comice' pears, respectively. Ethoxyquin at 1000ppm mixed in wax coating slows down chlorophyll degradation and reduce scuffing expression of 'Comice' pear.

The pre-mixed formulation of Difenoconazole + Fludioxonil (Syngenta product) applied as drenching at 16 oz. per 100 gallons control blue and gray mold decays at levels equivalent to Penbotech or Scholar alone. The different modes of action between Difenoconazole and Fludioxonil in the pre-mix may retard resistance development in the pathogens.