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The effect of pre-harvest ARMOUR-Zen® treatments on the development of postharvest botrytis rots in 'Sweetheart' cherries

Colhoun K, Elmer PAG, Stanley CJ, Attfield B

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Report approved by:

Kate Colhoun
Research Associate, Insect Behaviour and Ecology
May 2015

Louise Malone
Science Group Leader, Applied Entomology
May 2015

This report has been prepared by The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research).
Head Office: 120 Mt Albert Road, Sandringham, Auckland 1025, New Zealand, Tel: +64 9 925 7000, Fax: +64 9 925 7001.
www.plantandfood.co.nz

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EXECUTIVE SUMMARY

The effect of pre-harvest ARMOUR-Zen® treatments on the development of postharvest botrytis rots in 'Sweetheart' cherries

Colhoun K[1], Elmer PAG[2], Stanley CJ[1], Attfield B[1]
Plant & Food Research: [1]Clyde, [2]Ruakura

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Background

ARMOUR-Zen® is a natural product specifically developed for the control of *Botrytis cinerea* in wine grapes and ornamentals and *Sclerotinia sclerotiorum* in ornamentals. ARMOUR-Zen is manufactured and marketed by BotryZen (2010) Ltd, who wish to extend the range of application of this product. The primary aim of this project was to provide BotryZen (2010) Ltd with field-based evidence that pre-harvest ARMOUR-Zen treatments had efficacy against postharvest botrytis rots, thereby providing the cherry sector with a nil-residue alternative to the pre-harvest fungicide, Pristine®. The secondary aim was to determine if a pre-harvest spray programme, which integrated ARMOUR-Zen with synthetic fungicides, was as effective as a synthetic fungicide-only spray programme.

Methods and materials

A field-based trial was carried out on 'Sweetheart' cherries in a small (<1 ha) block leased to a neighbouring grower, located at the Clyde Research Centre, Central Otago from 19 December 2014 to 11 January 2015.

Four late-season spray programmes were evaluated for their efficacy against postharvest botrytis rot development. These four treatments were: 1) an untreated control, 2) a grower standard programme, 3) an integrated ARMOUR-Zen and fungicide programme and 4) an ARMOUR-Zen only programme.

Key results

All three treatments (grower standard, integrated programme and ARMOUR-Zen only) significantly reduced the development of postharvest botrytis rots over time, compared with the untreated (Nil) control.

There were no statistically significant differences between the rot incidences in the three different spray programmes evaluated.

Recommendations

Based on the evidence to date, ARMOUR-Zen shows promise for effective treatment of botrytis rots in cherries, and a range of further trials is recommended to fully demonstrate and quantify this. We recommend trialling ARMOUR-Zen in larger-scale field trials using commercial air-blast sprayers over several economically important cherry cultivars in Central Otago. In order for ARMOUR-Zen to be fully tested as a potential replacement for synthetic fungicides, it is recommended that field trials be established to evaluate further the costs and benefits of integrated and stand-alone ARMOUR-Zen programmes, and to determine the number of applications required for effective control of postharvest botrytis rots.

Recent reports of 'disastrous' disease control failures in Florida strawberry fields (Amiri et al. 2013) due to the emergence of resistance in populations to Pristine has highlighted the need for alternative products for botrytis rot control in New Zealand. A more extensive comparison of integrated programmes incorporating the use of both ARMOUR-Zen and synthetic fungicides with synthetic fungicide-only programmes is also recommended, along with monitoring of the change in sensitivity of the *B. cinerea* populations to the active ingredients in Pristine over several seasons to determine if integrated programmes can slow down the emergence of Pristine-resistant sub-populations. Pristine is a fungicide heavily relied upon by cherry growers and the demonstration of the benefits of an integrated programme would encourage growers to use ARMOUR-Zen in an integrated programme for botrytis control.

More detailed measurements of fruit maturity and other fruit quality attributes are also required (e.g. fruit firmness and soluble solids contents) to ensure there are no detrimental effects of ARMOUR-Zen applications on fruit maturity and quality.

For further information please contact:

Kate Colhoun
Plant & Food Research Clyde
990 Earnscleugh Road
RD1
Alexandra 9391
NEW ZEALAND
Tel: +64 3 904 7300
DDI: +64 3 904 7310
Fax: +64 3 904 7301
Email: kate.colhoun@plantandfood.co.nz

1 INTRODUCTION

ARMOUR-Zen® is a natural product fungicide (active ingredient = chitosan) that has been developed by BotryZen (2010) Ltd. It is currently registered in New Zealand as a control agent for *Botrytis cinerea* in wine grapes, ornamentals and cut flowers, and against *Sclerotinia sclerotiorum* in ornamentals.

Sweet cherry is a valuable export crop that relies upon the use of synthetic fungicides to protect the fruit against postharvest storage rots. The primary postharvest pathogen affecting sweet cherry production in Central Otago is *B. cinerea*. Other postharvest pathogens have been isolated, including *Monilinia* spp., *Alternaria alternata* and *Penicillium* spp., but these are not considered commercially important.

Widespread resistance to carbendazim and iprodione was detected in *B. cinerea* populations in Central Otago cherry blocks (Elmer et al. 2012; Elmer et al. 2014) and products containing these active ingredients have largely been replaced with Pristine®, a relatively new fungicide containing two active ingredients (pyraclostrobin and boscalid). The potential for development of resistance to Pristine, plus increasing pressure from overseas markets for fruit with nil to low pesticide residues, has highlighted an urgent need for alternative botrytis control treatments. A review carried out for Summerfruit NZ Incorporated (SNZ) identified four biologically based treatments with good efficacy against botrytis rots when tested on cherries in laboratory-based assays (Elmer et al. 2012). One of the treatments was the chitosan-based product, ARMOUR-Zen.

A request was received from BotryZen (2010) Ltd to establish a field trial and evaluate the efficacy of pre-harvest ARMOUR-Zen treatments against postharvest botrytis in a commercial block of 'Sweetheart' cherries. The primary aim of this project was to provide BotryZen (2010) Ltd with field-based evidence that pre-harvest ARMOUR-Zen treatments had efficacy against postharvest botrytis rots, thereby providing the cherry sector with a nil-residue alternative to the pre-harvest fungicide, Pristine. The secondary aim was to determine if a pre-harvest spray programme, which integrated ARMOUR-Zen with synthetic fungicides, was as effective as a synthetic fungicide-only spray programme.

2 METHODS

A field trial was established on 19 December 2014 in a commercial block of mixed cherry cultivars located at the Clyde Research Centre. This block was leased to a neighbouring orchardist and all trees received a conventional spray programme of recommended fungicides from flowering through to the beginning of the trial in mid December. A summary of treatments applied to the block of 'Sweetheart' trees are described in detail in Table 1 below. Each plot was at least one tagged branch on a single tree, and there were five replicate trees allocated in a randomised block design.

Table 1. Pre-harvest treatments, actions carried out, and the rationale for fungicide treatments applied to 'Sweetheart' cherries in Central Otago from 19 December 2014 to 11 January 2015.

Treatment	Actions	Rationale
Untreated from start of trial	Tagged branch was bagged just prior to the grower's spray application, then removed soon after.	Untreated control to show the development of postharvest botrytis rots when no treatment was applied pre-harvest.
Grower spray programme	Tagged branch received the grower's standard spray programme (no bagging required).	For comparison with the integrated and ARMOUR-Zen only programme.
Integrated programme	One of the three scheduled fungicides was replaced with ARMOUR-Zen by bagging the branch during the grower's spray application. The fruit on the tagged branch were then treated with ARMOUR-Zen later on the same day.	To demonstrate if an integrated programme was as effective as the grower's standard fungicide programme.
ARMOUR-Zen® only	The tagged branch was bagged during each grower spray application. The bag was then removed and the fruit treated with ARMOUR-Zen later on the same day.	To provide evidence that ARMOUR-Zen has efficacy against postharvest botrytis.

Between one and four branches on each tree were tagged to ensure there were no less than 200 fruit per replicate tree for sampling and postharvest analyses. All tagged branches were enclosed with either large black (1130 x 1470 mm) or white (600 x 400 mm) plastic bin liner bags, depending on the size of the tagged branch, immediately before the grower applied his standard spray treatment to the block with a conventional air-blast sprayer (Table 2). All bags were tied at the ends to prevent spray entry and were removed from each tagged branch c. 1 hour after the grower's spraying operations, and all ARMOUR-Zen treatments (10 mL/L) were then applied to just before run-off using a hand pressured 15-L (Croplands Swissmex) backpack sprayer. The only exception was the ARMOUR-Zen treatment on 1 January 2015, which had to be re-applied following rainfall.

Table 2. Treatment dates and fungicidal products applied to 'Sweetheart' cherries in the three-week preharvest period in Central Otago in 2014-15.

Treatment	22 Dec. 2014	30 Dec. 2014	1 Jan. 2015	5 Jan. 2015	11 Jan. 2015	Total no. of sprays
Nil preharvest	Nil	Nil	Nil	Nil	Nil	0
Grower standard	Pristine	Rapid	Nil	Corona	Nil	3
Integrated programme	Pristine	AZ	AZ	Corona	Nil	4
ARMOUR-Zen	AZ	AZ	AZ	AZ	AZ*	5

Where:

* An extra spray was applied to the ARMOUR-Zen (AZ) only programme at the request of the client on the day of harvest, to ensure there was good protective cover of the fruit in coolstorage, as it was five days since the last application. The additional benefit of the AZ spray is that it can be applied close to harvest.

AZ = ARMOUR-Zen®, a chitosan-based natural product fungicide applied at 10 mL/L

Pristine® = a fungicide with two active ingredients (pyraclostrobin and boscalid) applied at 50 g/100 L

Rapid™ 500 = a fungicide (active ingredient = iprodione) applied at 75 mL/100 L

Corona® 250 WG = a fungicide (active ingredient = tebuconazole) applied at 40 g/100 L

A sample of 100 blemish-free, harvest mature export grade fruit from each tree was picked on 11 January 2015, a day before the commercial harvest of the 'Sweetheart' fruit, and then placed into 55-count Plix® trays (50 fruit per tray). An extra clean Plix tray was inverted over the fruit and the two Plix trays from each tree were placed into a plastic fruit crate. The crates were stacked in the coolstore at 0°C until 30 January 2015 and then moved into the laboratory.

After removal from the coolstore, all fruit were assessed visually for postharvest rots and then enclosed in a clear plastic bag to maintain high relative humidity and incubated at 20°C with natural light. Each tray was again checked for rot development after three, six and twelve days of simulated 'shelf-life'. If any postharvest rots were found, each fruit was removed from the tray, placed in small plastic pottles (35 mL) that were sealed to prevent secondary spread, and sent to Plant & Food Research, Ruakura for rot identification.

2.1 Statistical analysis

Linear mixed models (LMM) were fitted to the data using the ASREML package (Butler et al. 2009) for R 2.15.0 (R Development Core Team 2012). Random effects were replicates (trays were split plots), and treatment was a fixed effect. Because the change in number of cherries infected with botrytis rots was a repeated measure, days of shelf-life could not be used as a fixed effect. Instead, the area under the disease progression curve (AUDPC) for each treatment was calculated by plotting the mean number of botrytis rots that had developed after 'shelf-life' periods of three, six and twelve days. Graphs with the AUDPC are presented in the Appendix.

3 RESULTS

The development of botrytis after coolstorage was significantly higher in the untreated control than in any of the other three treatments (Figure 1, P = 0.067).

There were no significant (P>0.05) differences in botrytis development between the grower's standard programme, the integrated programme, and the nil-residue ARMOUR-Zen only programme (Figure 1).

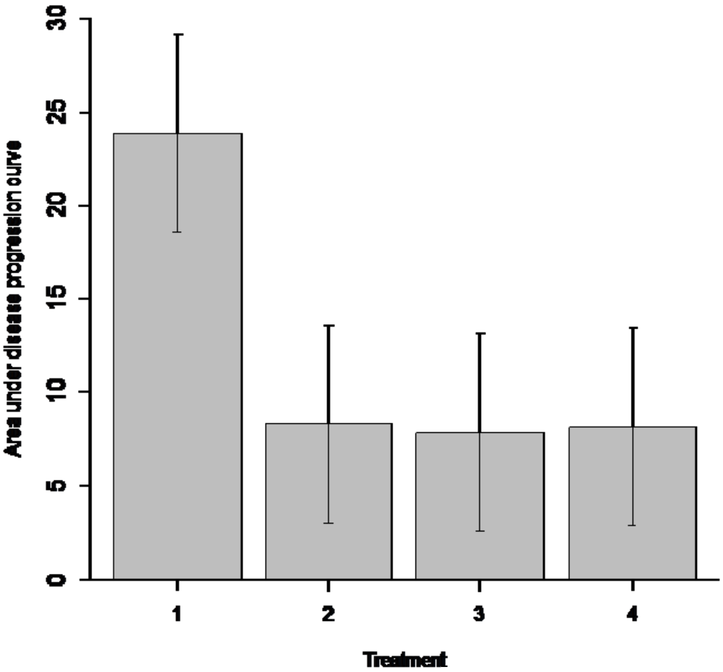

Figure 1. The effect of four pre-harvest fungicidal spray programmes on postharvest botrytis rot development in 'Sweetheart' cherries in Central Otago after three weeks of coolstorage at 0°C followed by up to 12 days simulated shelf life in laboratory conditions at 20°C, 2014-15. Treatment means are the average values for the area under the disease progress curve (AUDPC) for each treatment programme. Error bars represent the standard errors of the mean.

Treatment 1 = Nil preharvest treatment, Treatment 2 = Grower's standard preharvest fungicide programme used for botrytis control, Treatment 3 = Integrated programme, Treatment 4 = ARMOUR-Zen® only

4 CONCLUSION AND RECOMMENDATIONS

This preliminary field trial showed that the ARMOUR-Zen only treatment was effective at reducing postharvest botrytis rots in 'Sweetheart' cherries. Importantly, the ARMOUR-Zen only programme (n = 5 sprays) gave botrytis rot control that was equal to that of the grower's standard programme, which consisted of three synthetic fungicides.

In this trial, five applications of ARMOUR-Zen (Treatment 4) were made in the three weeks leading up to harvest. While this meant that there was good coverage on the ARMOUR-Zen treated fruit, it may be argued that this number of applications may not be cost-effective.

This was a small-plot field trial with treatments applied by backpack sprayer to individual shoots. In order to test the effectiveness of ARMOUR-Zen fully, as a possible replacement for synthetic fungicides, or as a treatment to be used in integrated programmes to reduce the number of synthetic fungicide applications, it is recommended that field trials be established to evaluate further the costs and benefits of integrated and stand-alone ARMOUR-Zen programmes, and to determine the number of applications required for effective control of postharvest botrytis rots. Larger-scale field trials using commercial air-blast sprayers are also recommended.

Recent reports of 'disastrous' disease control failures in Florida strawberry fields (Amiri et al. 2013) due to the emergence of resistance in *B. cinerea* populations to Pristine highlight the need for alternative products for botrytis rot control in New Zealand. The integration of a product like ARMOUR-Zen into recommended spray programmes would reduce the number of Pristine applications, and may reduce the selection pressure on *B. cinerea* populations. A reduction of selection pressure may extend the effective commercial life of valuable new fungicides, such as Pristine. Therefore, it is also recommended that further field testing of integrated ARMOUR-Zen and synthetic fungicide programmes, compared with synthetic fungicide programmes only, be carried out. This would require monitoring of *Botrytis* populations for sensitivity changes to the active ingredients in Pristine on a regular basis over several seasons, to determine if integrated programmes slow down the emergence of Pristine-resistant sub-populations. However, this is beyond the scope of this current project, and financial assistance from relevant funding agencies (e.g. the Ministry for Primary Industries' Sustainable Farming Fund) and stakeholder groups (e.g. Summerfruit New Zealand and chemical companies) would greatly assist a project of this nature.

5 ACKNOWLEDGEMENTS

We would particularly like to thank the orchardist, Murray Miller for letting us run the trial in his leased block and for his cooperation and communication on the timing of his spray programme. Many thanks to Ross Owens and Claire Scofield for applying the ARMOUR-Zen spray treatments to the field trial. Thanks to Peter Alspach for valuable advice on the statistical analyses. We also thank Claire Hall, Louise Malone and Anne Gunson for helpful editorial suggestions.

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APPENDIX

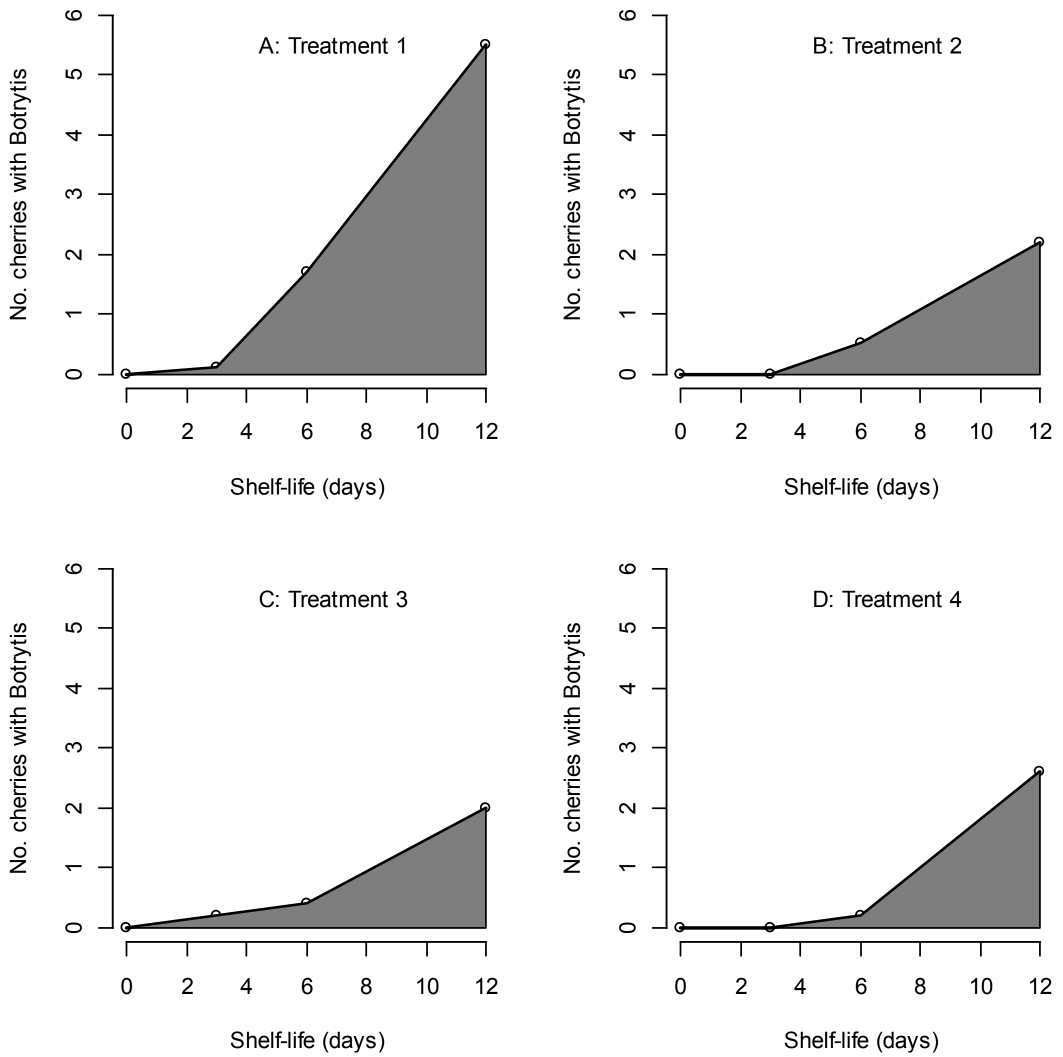

Figure A1. The effect of pre-harvest fungicidal spray programmes on the mean number of 'Sweetheart' cherries in Central Otago infected with botrytis rots after harvest on 11 January 2015, and coolstorage at 0°C for 14 days, followed by incubation on the laboratory bench for 3, 6 and 12 days. The shaded area for each treatment represents the area under the disease progress curve (AUDPC).

Treatment 1 = Nil preharvest treatment, Treatment 2 = Grower's standard preharvest fungicide programme used for botrytis control, Treatment 3 = Integrated programme, Treatment 4 = ARMOUR-Zen® only

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