

CP 205 AHDB Horticulture Efficacy Trials 2022

Final Trial Report

Work package:	WP 19
Title:	Testing biological and conventional products as post-harvest treatments to reduce brown rot and other storage losses in sweet cherry
Crop	Cherry, <i>Prunus avium</i>
Target	Post-harvest brown rot (<i>Monilinia laxa</i> , <i>Monilinia fructigena</i>) and other post-harvest rots
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Organisation:	NIAB East Malling
Period:	Aug 2022- Oct 2022
Report date:	20/11/22
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ORETO 411	Trial done in accordance to ORETO standard. Certificate in appendix

I the undersigned, hereby declare that the work was performed according to the procedures herein described and that this report is an accurate and faithful record of the results obtained

Date

Author's signature

Trial Summary

Introduction

The objective of this trial was to test if post-harvest dipping of cherry fruit in solutions of different fungicides, biocontrol agents and sterilant products could reduce post-harvest rot in cherry. We had particular emphasis on brown rot control caused by *Monilinia* species.

Methods

Cherry fruit (cv Sweetheart) was sourced from commercial orchard and transported to NIAB East Malling within 6 h of picking on 03/08/2022. The fruit was first randomised into 2 kg units, omitting any damaged / less than perfect fruit. Brown rot (*Monilinia laxa*, *Monilinia fructigena*) spores were harvested from mummified infected fruit collected from orchard at NIAB site. Fruit was inoculated by dipping into spore suspension for 30s followed by ca. 20 minutes at ambient temperature (ca. 27°C) before and moving to overnight storage at 4°C. The following day the fruit was dipped in product suspensions for 30 s and drained / dried at ambient temperature for 30 min. Two control treatments/products were used. Product 1 was a non-inoculated, untreated control (fruit as delivered from the orchard) which served to gauge the levels of natural infections on the fruit. Product 2 was a brown rot inoculated, water treated control to which all products were compared to. Fruit was packed into modified atmosphere bags and stored at +1 - +2°C and 99% humidity for 18 days. Each product was tested in five replicates each replicate consisting of 2 kg of treated fruit.

Fruit was assessed for post-harvest rots immediately upon 18 days of cold storage and again after 8 days of incubation at ambient temperature and 95% humidity. At each assessment the fruit was separated into 6 categories: 1) healthy / marketable fruit, 2) *Monilinia* (brown rot) infected fruit, 3) *Botrytis* (grey mould) infected fruit, 4) *Penicillium* (blue mould) infected fruit, 5) fruit with other visible rots (*Cladosporium*, *Mucor*, *Fusarium*, etc) and 6) other symptoms, such as damaged unmarketable fruit with no visible rots. The taxonomic classification of each rot category was confirmed using PCR and Sanger sequencing of internal transcribed region 1 (ITS1).

Results

Over 95% of fruit was categorised as healthy immediately after 18 days of cold storage regardless of the treatment (figure 1). *Bacillus* based biocontrol product 6 was the only treatment with a slightly, but significantly, higher proportion of healthy fruit (~98%) than the inoculated control (~97.3%). *Trichoderma* based biocontrol product 7 was the only product with a slightly, but significantly, lower proportion of healthy fruit (~95%) than the inoculated control. Brown rot, grey mould and blue mould infected fruit was not identifiable or it was observed at frequency too low for statistical analysis. Most of the unmarketable fruit was due to unidentifiable rots or other symptoms. This indicated that good storage conditions (low temperature, modified atmosphere bags) can inhibit rot development during cold storage even when very high inoculum levels are present on the fruit.

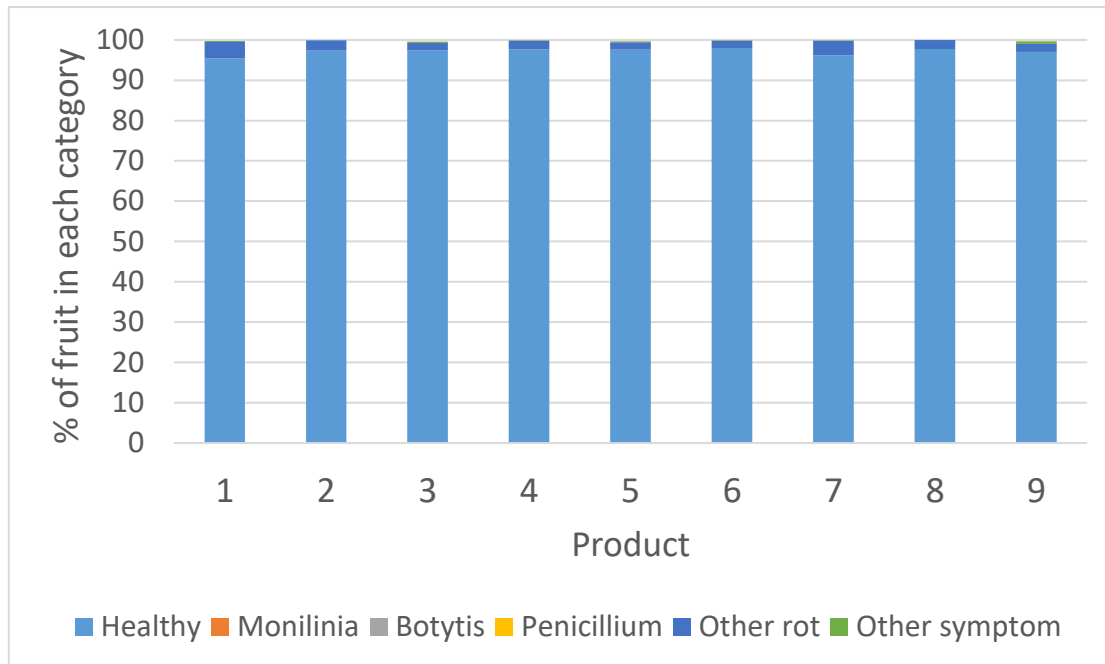


Figure 1. Proportion of fruit in each category immediately after 18 days of cold storage.

After 8 days of ambient temperature incubation we observed significant differences between treatment products in terms of post-harvest rot control. Less than 50% of fruit was left marketable in control treatments; in the non-inoculated control (product 1) most of the rots were in the other rots category while most of the rots in the inoculated control (product 2) were due to brown rot. Fungicide products 3 and 4 performed best with over 75% and 80% of fruit remaining marketable, respectively (figure 2). Product 3 significantly reduced *Monilinia*, *Botrytis*, *Penicillium* and other symptoms but not the other rots. Product 4 significantly reduced *Monilinia*, other rots and other symptoms, but not *Botrytis* or *Penicillium* rots. Pesticide residue analysis of fruit treated with product 3 and 4 have not found any active ingredient above MRL levels. We therefore recommend products 3 and 4 for EAMU for post-harvest control of fruit rot in cherry.

All tested biocontrol products increased proportion of healthy fruit after 8 days of ambient temperature incubation in comparison to the inoculated control, but to a lesser degree than fungicides. Products 6 and 8 maintained ~65% and product 5 and 7 between 55% and 60% of healthy fruit after 8 days of ambient temperature incubation. Efficacy of biocontrol products also varied greatly between different rots. Product 5 significantly reduced *Monilinia* and other symptoms, less effectively reduced *Penicillium* rots and did not reduce *Botrytis* or other rots. Product 6 reduced *Monilinia*, *Botrytis*, other rots and other symptoms but significantly increased rots due to *Penicillium*. Product 7 reduced *Monilinia* and other symptoms, but increased *Botrytis*, *Penicillium* and other rots. Product 8 reduced *Monilinia* infections, other rots and other symptoms but had no effect on *Botrytis* or *Penicillium* infections. These results indicate that the biocontrol products would be most useful in orchards where *Monilinia* is the main concern and *Botrytis*, *Penicillium* or other rots pose low risk. Selection of the most effective biocontrol product for each orchard will need to be done with the knowledge of the risks for each specific rot type. The risks could be

identified by ambient temperature incubation of a representative sample of fruit followed by rot assessment prior to the main harvest. Based on this data it would be useful if EAMUs could be secured for all four biocontrol products for post-harvest use in cherry so the growers and advisors could choose the best product for their circumstances. The only product with negligible effect on post-harvest rot was sterilant product 9.

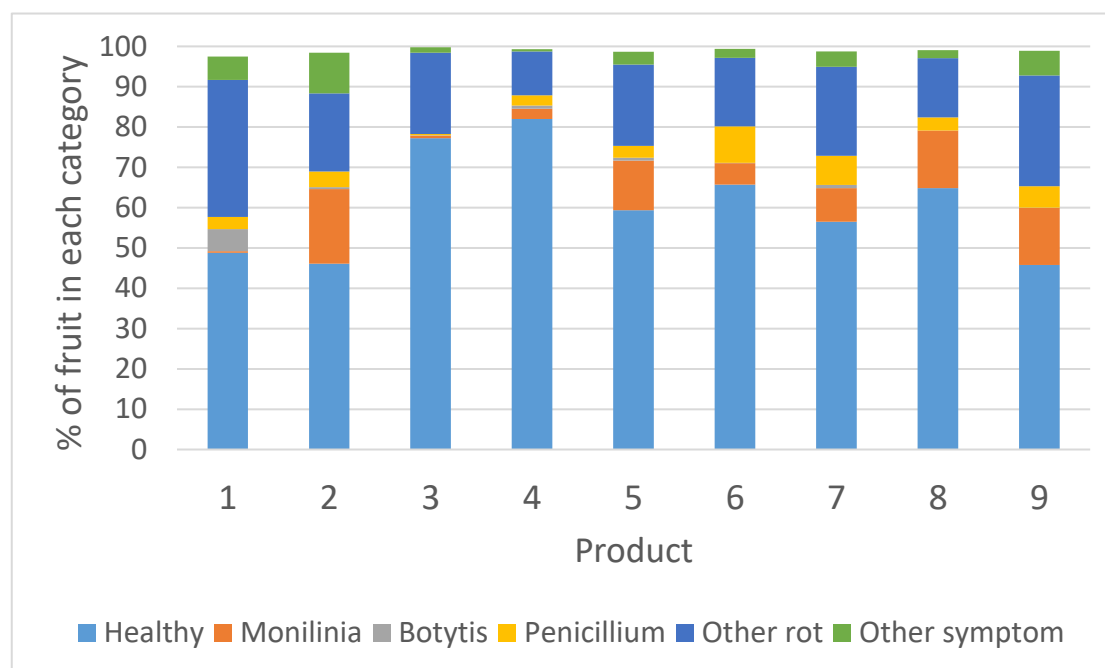


Figure 2. Proportion of fruit in each category immediately after 18 days of cold storage and 8 days of room temperature incubation.

Conclusions

Low temperature storage (max +2°C) in modified atmosphere bags can almost completely stop cherry post-harvest rot development during 18 days of storage even in the scenario of very high inoculum levels used in this study.

All but the sterilant product significantly improved the percent of marketable fruit at 18 days of cold storage followed by 8 days of room temperature incubation compared to untreated control.

Overall the best products for protection of cherry against post-harvest rots were fungicide products 3 and 4.

All biocontrol products significantly increased percent of healthy fruit and reduced *Monilinia* rot, but to a lesser degree than fungicides. The best biocontrol products were product 6 and 8 followed by 5 and 7.

Importantly, the efficacy of biocontrol products and to lesser degree fungicides differed depending on the specific rot in question (Table 1). It is therefore important to assess the risk of different rots in the orchard to pick the product that is most appropriate for use in specific circumstances.

Take home message:

- Appropriate cold storage conditions (<+2°C and modified atmosphere) will prevent development of post-harvest rots during cold storage.
- Post-harvest dipping in the two fungicide products 3 and 4 can significantly reduce post-harvest rots on cherry even in a high inoculum scenario without breaching any pesticide residue MRLs.
- All four tested biocontrols have shown control of *Monilinia* and other symptoms but were more variable in the case of *Botrytis*, *Penicillium* and other rots. Biocontrol products may be more effective in lower disease pressure scenario.
- Results also indicated that some biocontrol products may increase certain post-harvest rots. Caution must be taken to assess the risk of various rots in the orchards and use specific biocontrol products when the fruit is at high risk of *Monilinia* but at much lower risk of other rots.

Table 1. Summary of product effectiveness against different post harvest rots measured in the study. Very effective products reduced post harvest rot by more than 75% (compared to inoculated control); moderately effective products reduced by 50-75% and slightly effective products reduce post harvest rots by 10-50%.

Prod. No.	Product Type	AHDB code	Brown rot (<i>Monilinia spp.</i>)	Grey mould (<i>Botrytis cinerea</i>)	Blue mould (<i>Penicillium spp.</i>)	Other moulds (<i>Mucor</i> , <i>Cladosporium</i> , <i>Alternaria</i> , <i>Fusarium</i>)
3	Fungicide	AHDB 9704	Very effective	Very effective	Very effective	Not effective
4	Fungicide	AHDB 9816	Very effective	Not effective	Slightly effective	Moderately effective
5	Biocontrol	AHDB 9791	Slightly effective	Not effective	Not effective	Not effective
6	Biocontrol	AHDB 9936	Moderately effective	Very effective	Not effective	Slightly effective
7	Biocontrol	AHDB 9788	Moderately effective	Not effective	Not effective	Not effective
8	Biocontrol	AHDB 9758	Slightly effective	Very effective	Not effective	Slightly effective
9	Sterilant	AHDB 9703	Slightly effective	Very effective	Not effective	Not effective

SCIENCE SECTION

Objectives

The objective was to assess if a range of fungicides, biological control agents and sterilant products applied to cherry fruit as a post-harvest dip could reduce storage rots, in particular brown rot caused by *Monilinia spp.*

Methods

Trial conduct

The following EPPO guidelines were followed:

Relevant EPPO guideline(s)		Variation from EPPO
PP1/152(4)	Design and analysis of efficacy evaluation trials	/
PP1/181(5)	Conduct and reporting of efficacy evaluation trials, including good experimental practice	/
PP1/223(2)	Introduction to the efficacy evaluation of plant protection products	/
PP1/214(4)	Principles of acceptable efficacy	/
PP1/239(3)	Dose expression for plant protection products	/

Test site

Item	Details
Location address	NIAB East Malling, New Road, East Malling
Crop	Sweet cherry fruit
Cultivar	Sweetheart
Soil or substrate type	Not relevant
Agronomic practice	Fruit grow using a standard commercial practice
Prior history of site	According to the agronomists, the grower that provided the fruit had a history of high post-harvest rot incidence.

Trial design

Item	Details
Trial design:	9 treatments in fully randomised block design
Number of replicates:	5
Row spacing:	Not applicable
Plot size: (w x l x h)	Crate size 30 x 20 x 10 cm
Plot size:	2 kg of fruit in individual crate
Number of plants per plot:	Ca. 200 fruit per crate
Leaf Wall Area calculations	Not applicable

Fruit sourcing

- 1) Cherry fruit cv Sweetheart fruit was sourced from a commercial producer from Worcestershire.
- 2) The fruit was picked into standard cherry crates and transported to NIAB within 6h of picking.
- 3) On arrival to NIAB East Malling the fruit was checked for rots and any other visual deformations. Only immaculate fruit was selected for the trial
- 4) Fruit was randomised into 55 smaller crates each holding 2 kg of fruit. Each 2 kg crate served as one experimental unit.
- 5) The fruit was stored at 4°C overnight until inoculation.

Brown rot inoculation

- 6) Mummified, brown rot infected fruit was collected from a cherry orchard on NIAB site on the day of inoculation.
- 7) Fruit with *M. laxa* and *M. fructigena* symptoms was used in approximately 1:1 ratio.
- 8) Infected fruit was mixed with distilled water and a drop of Tween 20 in 2 L flasks and shaken for 30 min at room temperature to collect the spores.
- 9) Spore suspension was filtered through double layer of clean muslin cloth, enumerated using standard haemocytometer methodology and used on the day of collection at a final concentration of 3.57×10^4
- 10) Inoculation was done by submerging each experimental unit (2kg fruit in a plastic mesh crate) in 20 l of *Monilinia spp.* spore suspension for 30 s
- 11) Fruit was drained and dried for 20 mins at air temperature (ca. 27°C) before storing it at 4°C overnight until product application the next day

Test product application

- 12) 18-22h after inoculation the fruit was brought out of overnight 4°C cold store and left at ambient temperature (24-26 °C) for 1-2 h before treatment application.
- 13) Treatments listed in table 2 were applied according to application details in table 3. Rates were calculated based on maximum single dose and minimum single application volume stated in each product label.
- 14) Fruit was submerged in water solution of products for 30 s (volume as stated in table 3) with gentle agitation.
- 15) Fruit were left to drain and dry for 1h at ambient temperature before storage.

Storage

- 16) Fruit from every experimental unit was stored in a separate modified atmosphere bag designed for optimal storage of cherry fruit (Cherry 902 plus, View Fresh).
- 17) A Datalogger recoding temperature and relative humidity was placed inside bags.
- 18) Bags were twisted, folded and secured using elastic bands and gently positioned in individual cardboard storage crates.
- 19) Crates were stacked on a pallet and blocked according to the position on the pallet i.e., crates in block 1 were randomized in the first two rows, crates from block 2 were randomised in the second two rows, etc.
- 20) Fruit was stored at 2°C and 100% humidity in the bag for 18 days.

Assessment

- 21) There were no post-harvest rots present at treatment application.
- 22) First fruit assessment was done at day 0 after cold storage (day 18 after treatment). Fruit from each experimental unit was separated into 6 categories (listed below) based on visual symptoms (see Appendix: Trial Photographs) and the weight of each category measured:
 1. Healthy / marketable fruit
 2. *Monilinia* (brown rot) symptoms
 3. *Botrytis* symptoms
 4. *Penicillium* symptom
 5. Other rots including multiple or visually unidentifiable rots
 6. Other damage (damaged unmarketable fruit with no distinct rots)
- 23) Only fruit that was visually healthy at day 0 was incubated further at room temperature (22-24°C) in modified atmosphere bag and high humidity (96-99% RH). Rotten fruit was discarded.
- 24) Rots were monitored daily until a significant amount of rotten fruit was observed in inoculated control (product 2).
- 25) Second assessment was conducted at day 8 after storage.

Rot taxonomy confirmation

- 26) Five representative samples of fruit from each rot category were sampled for post-harvest rot confirmation via PCR and Sanger sequencing:
- 27) DNA was extracted directly from sporulating fungi on the fruit using Sigma Extract'n'Amp kit.
- 28) ITS1 region of the DNA was amplified using primers developed by White et al. (1990) followed by forward and reverse Sanger sequencing using the same set of primers.
- 29) Forward and reverse reads were trimmed by sequencing quality and de-novo aligned in Geneious Prime (v2022.0.1) software.
- 30) Consensus sequences were used for NCBI BLAST search (blastn) to determine the most likely taxonomy of each sample.

Pesticide residue analysis

- 31) At second assessment (day 8 after storage) a sample (500g) of healthy fruit was pooled across 5 plots per treatment.
- 32) Commercial standard (product 2) and fungicide treatments (product 3, 4) were sampled.
- 33) Fruit was sent to external certified lab (Tentamus QTS Analytical Ltd) for detection of pesticide residues.

Treatment details

Table 2. List of treatments.

Prod. No.	Product Type	Inoculated	AHDB code	Serial number and Date of manufacture
1	Negative control	NO	\	\
2	Commercial standard	YES	\	\
3	Fungicide	YES	AHDB 9704	C04D22; 04/2022
4	Fungicide	YES	AHDB 9816	POROJ50072; 10/2020
5	Biocontrol	YES	AHDB 9791	EG257581; expiry date 12/2023
6	Biocontrol	YES	AHDB 9936	EMBI000147; 20/2022
7	Biocontrol	YES	AHDB 9788	EBAB006157; 11/2021
8	Biocontrol	YES	AHDB 9758	05.2022-YCF046; 05/2022
9	Sterilant	YES	AHDB 9703	No details available

Application schedule

Table 3. Application details

Prod. No.	Product Type	Inoculation?	AHDB code	Water volume (L)	Product amount	Unit
1	Negative control	NO	\	\	\	\
2	Commercial standard	YES	\	20	\	\
3	Fungicide	YES	AHDB 9704	20	100	g
4	Fungicide	YES	AHDB 9816	20	30	g
5	Biocontrol	YES	AHDB 9791	20	41.1	g
6	Biocontrol	YES	AHDB 9936	20	800.0	ml
7	Biocontrol	YES	AHDB 9788	15	30.0	g
8	Biocontrol	YES	AHDB 9758	20	80	g
9	Sterilant	YES	AHDB 9703	20	857	ml

Application details

Task / Parameter	Application
Fruit picking date	03/08/22
Inoculation date	03/08/22
Application date	04/08/22
Application time of day	10am to 3pm
Crop growth stage (Max, min average BBCH)	Fruit
Crop height (cm)	Not applicable
Crop coverage (%)	100
Application Method	Fruit dipping in treatment solution
Application Placement	Not applicable
Application equipment	Not applicable
Nozzle pressure	Not applicable
Nozzle type	Not applicable
Nozzle size	Not applicable
Application water volume/ha	Not applicable
Storage interval (date)	04/08/22 - 22/08/22
Storage temperature (°C)	2
Relative humidity (%)	99-100
Wind speed range (m/s)	Not applicable
Dew presence (Y/N)	No
Temperature of soil - 2-5 cm (°C)	Not applicable
Wetness of soil - 2-5 cm	Not applicable
Cloud cover (%)	Not applicable
Assessment 1 (date)	22/08/22
Room temperature incubation interval (date)	22/08/22 – 30/08/22
Incubation temperature (°C)	22-24°C
Incubation relative humidity (%)	96-99 (Inside bag)
Assessment 2 (date)	30/08/22

Statistical analysis

Statistical data analysis was done in R studio (v2022.07.2) running R4.1.1 environment.

The proportion of fruit in each category (e.g. weight of healthy fruit / total weight of unmarketable fruit) was analysed at each assessment separately using generalised linear model with binomial distribution of residuals and logit link function. Block and Product were used as fixed factors. The significance of fixed factors was determined using ANOVA with Chi square test. Pairwise comparisons of treatments was made in package “emmeans” (Russell, 2022) with Tukey adjusted p-values.

The data from the first assessment (0 days at room temp after storage) was used unchanged, whereas the data from the second assessment (8 days at room temp after storage) was converted into cumulative (sum of both assessments) to more accurately present the total losses due to each rot and the remaining healthy fruit at the end of the experiment.

Results

Healthy fruit

Both Block ($df=4$, $p(\text{Chi})<0.001$) and Product ($df=8$, $p(\text{Chi})<0.001$) had significantly affected % of healthy fruit at day 0 and day 8 assessments. Differences between blocks were small but significant; less than 1% and 5% at day 0 and day 8 assessment, respectively (Supp table 1).

At the point of taking fruit from cold storage (day 0) all treatments had between 95% and 98% healthy, marketable fruit (figure 3 A). The differences between treatments at day 0 were small, less than 3%. Untreated, uninoculated control (product 1) and biocontrol product 7 were the only products with significantly less healthy fruit than inoculated untreated control (product 2). Biocontrol product 6 had slightly but significantly more healthy fruit than control (product 2).

After 8 days of room temperature incubation the percent of healthy fruit decreased to between 45% and 82% (figure 3, B). There were large and significant differences between products at day 8 (figure 3, B). As expected, non-inoculated, untreated control (product 1) had slightly but significantly higher percent of healthy fruit than inoculated untreated control (product 2), indicating the inoculation did increase disease pressure. Inoculated, untreated control (product 2) and sterilant product 9 had the lowest percent of marketable fruit at 8 days with 48% and 45 % of fruit remaining healthy, respectively. The product with the highest percent of marketable fruit at 8 days was fungicide product 4 with 82% of marketable fruit followed by fungicide product 3 with 77 %, biocontrol products 6 and 8 with ~65%, biocontrol product 5 with 59% and biocontrol product 7 with 56 % of marketable fruit.

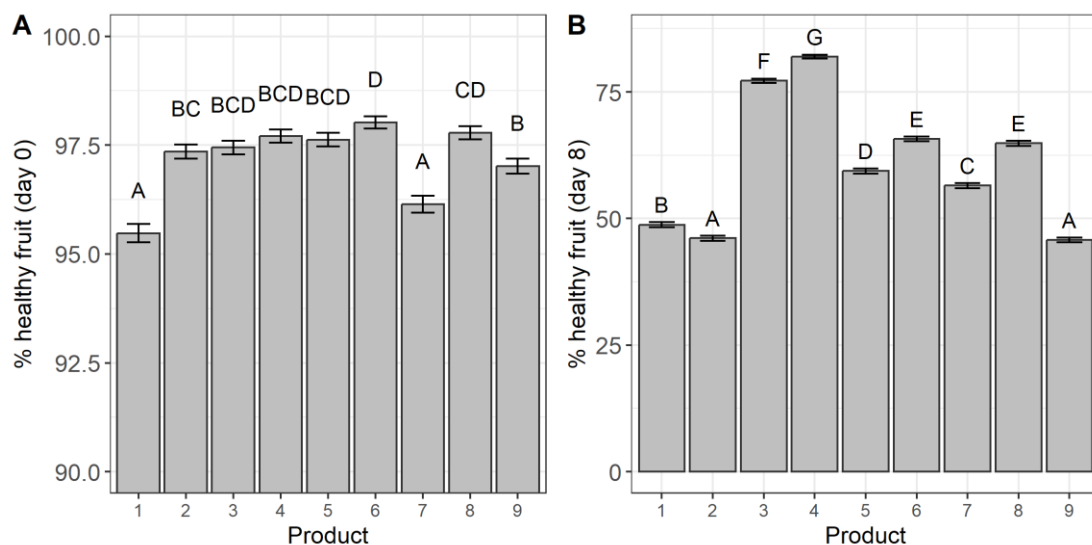


Figure 3. Mean percent (\pm SE) of healthy, marketable fruit: A) Immediately after 18 days of cold storage (day 0); B) After 18 days of cold storage followed by 8 days of room temperature incubation (day 8). Products 1 and 2 are non-inoculated and inoculated water treated controls, respectively. Groups denoted with different letters are considered statistically significantly different from each other based on Tukey's post-hoc test ($p<0.05$).

***Monilinia, Botrytis and Penicillium* infected fruit**

The ITS1 PCR and Sanger sequencing confirmed that the fruit visually categorized as infected with *Monilinia*, *Botrytis* and *Penicillium* were indeed infected with aforementioned fungi. Two *Monilinia* samples were taken from each of the 5 blocks. In each pair one was confirmed as *M. fructigena*, and the other *M. laxa*. For *Botrytis*, one isolate per block was sampled and confirmed as *B. cinerea* apart from block 5 which failed to sequence. One *Penicillium* isolate was sampled and tested per block and all five were confirmed as *Penicillium* genus with the highest similarity to *P. expansum* (NCBI Blast nt). See fasta sequences in Appendix: Sequencing data.

The rate of post-harvest rots caused by *Monilinia*, *Botrytis* and *Penicillium* at day 0 (immediately after 18 day of cold storage) were 0 or too low for statistical analysis (see Appendix: Means and standard errors).

After 8 days of room temperature incubation the percent of *Monilinia*, *Botrytis* and *Penicillium* infected fruit in untreated, inoculated control (product 2) increased to 18.5%, 0.5% and 3.9%, respectively (figure 4, A, B,C).

Both Block (df=4, p(Chi)<0.001) and Product (df=8, p(Chi)<0.001) had significantly affected % of *Monilinia*, *Botrytis* and *Penicillium* infected fruit at day 8. Differences between blocks were small but significant (Supp table 1).

Comparison of inoculated control (product 2) with non-inoculated (product 1) indicated that *Monilinia* inoculation was very successful with 18% of fruit infected in inoculated (product 2) and only 0.5% in non-inoculated control (product 1). Similarly, *Penicillium* levels were slightly but significantly higher in inoculated control (3.9%) compared to non-inoculated (3.1%). *Botrytis* levels however were below 1% in all treatments except for the untreated non-inoculated control (product 1) which had ~5.5% of fruit with *Botrytis* infections.

Treatment products had different effects depending on the post-harvest rot in question. Fungicide product 3 significantly reduced post-harvest rots of all three pathogens in comparison to inoculated control (product 2). It was the only product that reduced *Monilinia* infections to the level of non-inoculated control (product 1) and at the same time significantly reduced *Botrytis* and *Penicillium* levels below levels of both inoculated (product 2) and non-inoculated (product 1) control.

Product 4 (fungicide) reduced *Monilinia* and *Penicillium* infections compared to inoculated control (product 2) but the levels of both rots were equal or significantly higher than in non-inoculated control (product 1). Product 4 did not affect *Botrytis* infections.

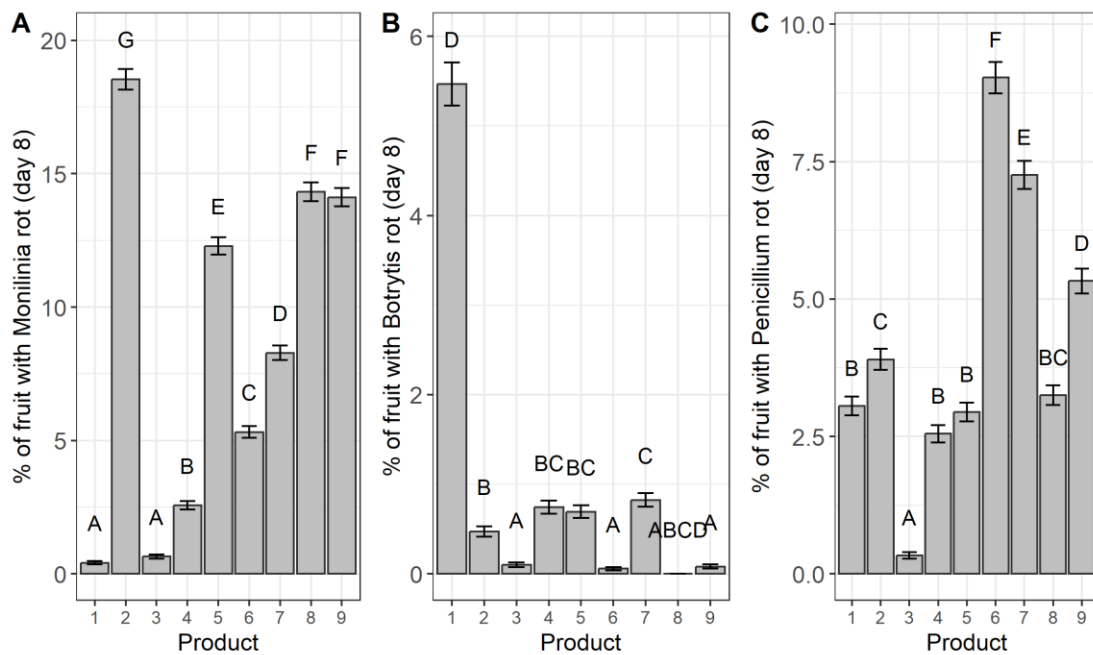


Figure 4. Mean percent (\pm SE) of fruit infected with: A) *Monilinia*, B) *Botrytis* and C) *Penicillium* measured after 8 days of room temperature incubation. Products 1 and 2 are non-inoculated and inoculated water treated controls, respectively. Groups denoted with different letters are considered statistically significantly different from each other based on Tukey's post-hoc test ($p < 0.05$).

Biocontrol products (product 5-8) all significantly reduced infections by *Monilinia* but the effect size varied greatly when comparing to the inoculated control which had 18% *Monilinia* infections. The most effective was product 6 (~5% *Monilinia* infections) followed by product 7 (~8% infections), product 5 (12% infections) and the least effective was product 8 with almost 15% of fruit with *Monilinia* rot.

Sterilant product 9 slightly, but significantly reduced *Monilinia* infections to below 15%.

Biocontrol products 6 and 8 and sterilant product 9 also significantly reduced the rate of *Botrytis* infected fruit in comparison to both inoculated (product 2) and non-inoculated (product 1) control. In the case of product 8, there was no *Botrytis* infected fruit detected and thus the model could not reliably assign statistical significance to that treatment (figure 4 b). Other products did not affect *Botrytis* infection rate, with exception of product 7 that slightly, but significantly increased infections compared to inoculated control (product 2).

The results on *Penicillium* however were very different. Only product 3 significantly reduced *Penicillium* levels to below inoculated (product 2) and non-inoculated (product 1) control. Products 4 and 5 slightly but significantly reduced *Penicillium* levels in comparison to inoculated control (product 2, 3.9% *Penicillium* infections). Products 6, 7 and 9 significantly increased *Penicillium* levels to above ~9%, ~7% and 5%, respectively.

Other rots and other symptoms

In each block we sequenced all visually distinct rots we found across all treatments and the results were as follows: in Block 1 we had *Mucor piriformis*, *Cladosporium sp.*, and *Alternaria sp.* rots. In block 2 we found *Fusarium sp.* and *Cladosporium sp.* In block 3 we had *M. piriformis*, *Cladosporium sp.* and *Fusarium sp.* rots. Block 4 had *M. piriformis*, *Fusarium sp.*, *Cladosporium sp.*, and *Trichoderma sp.* Block 5 had *M. piriformis*, 1 *Fusarium sp.*, and *Cladosporium sp.* rot.

Both Block (df=4, p(Chi)<0.001) and Product (df=8, p(Chi)<0.001) had significantly affected % fruit with other rots and other symptoms at day 0 and 8. Differences between blocks were small but significant (Supp table 1).

Similarly to *Botrytis* infections, the % of fruit with other rots was by far the highest in the non-inoculated, untreated control (product 1) with above 4% and 30% of fruit with other rots on day 0 and day 8, respectively (figure 5 a, b). This indicated that the act of inoculation with brown rot may have masked other rots, washed away spores or inhibited other fruit rots from developing to visually detectable levels.

Product 3 which was best at controlling *Monilinia*, *Botrytis* and *Penicillium* reduced other rots to below inoculated control (product 2) at day 0 but not at day 8. At day 0, only products 3, 5 and 6 slightly but significantly reduced other rots from above 2.5% in inoculated control to below 2%. Product 7 however, increased other rots at day 0 to above 3.5%.

At day 8, product 4 (~10% infections), 6 (~17% infections) and 8 (15% infections) significantly decreased other rots in comparison to inoculated control (product 2, 20% infections). Product 7 (~23% infections) and 9 (~28% infections) significantly increased other rots at day 8.

Less than 1 % of fruit had other symptoms, i.e. minor damage or pitting with no visible rots at day 0, and between 1% and 10 % at day 8. The data at day 0 was analysed but may not be of commercial importance due to extremely low levels of the other symptoms.

At day 8 however, all products decreased the levels of other symptoms to below levels of both controls (product 1 and 2) except for product 9, which reduced levels of other symptoms significantly below inoculated control but not in comparison to non-inoculated control (product 1).

Product 4 (0.5%) and product 3 (~1.5%) had the lowest levels of fruit unmarketable due to other symptoms, followed by products 6 and 8 with ~2.5% and products 5 and 7 with ~3.5% of other symptoms. Noteworthy, at day 8 inoculated control had significantly higher (product 2, ~10%) levels of other symptoms compared to non-inoculated control (product 1, ~6%) indicating that inoculation have not only increased visible *Monilinia* rots but also other damage that would make fruit unmarketable.

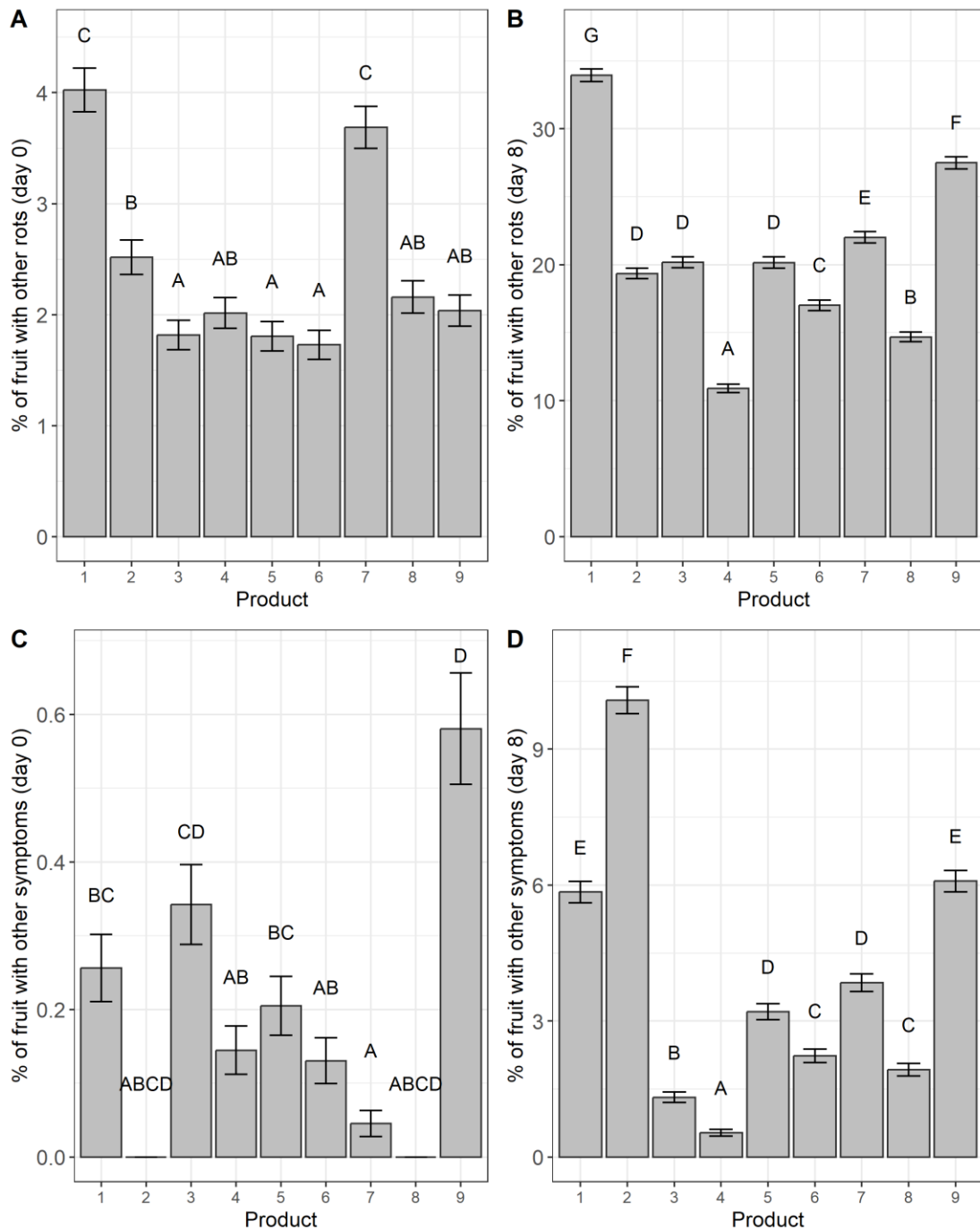


Figure 5. Mean percent (\pm SE) of fruit infected with: A) other rots at day 0; B) other rots at day 8; C) other symptoms (damage with no visible rot) at day 0; D) other symptoms at day 8. Products 1 and 2 are non-inoculated and inoculated water treated controls, respectively. Groups denoted with different letters are considered statistically significantly different from each other based on Tukey's post-hoc test ($p < 0.05$).

Pesticide residue analysis

Fruit treated with product 2 (inoculated control) and the two fungicide products (product 3 and 4) were tested for presence of pesticide residues. None of the samples exceeded any of the MRL values. See Appendix: Pesticide residue data for details.

Discussion

Control of cherry post-harvest rots during cold storage

The overall results of the assessment immediately after the 18 days of storage (day 0) indicated that good storage regime such as low temperature and appropriate modified atmosphere can reduce total storage rots to between 2- 5% even with very high inoculum levels used in this study. At day 0, most treatment products showed results indistinguishable from the inoculated control (product 2). *Monilinia*, *Botrytis* and *Penicillium* rots were not detected. The small fraction of unmarketable fruit immediately after cold storage was due to rots such as *Mucor sp.*, *Cladosporium sp.*, *Alternaria sp.*, *Fusarium sp.* and *Trichoderma*. Interestingly, the only product with significantly higher percent of healthy fruit and lowered percent of rots immediately after cold storage was *Bacillus* based biocontrol product 6. *Trichoderma* based biocontrol product 7 however, had significantly less healthy fruit and more other rots immediately upon storage compared to inoculated control (product 2). It is conceivable that *Trichoderma* may be able to grow at the storage conditions and cause this reduction of marketable fruit. It has to be pointed out however, that the percent of healthy fruit in product 7 was not lower than in non-inoculated control (product 1) which had the lowest percent of healthy fruit and the highest percent of other rots at day 0.

Control of post-harvest rots after cold storage and room temp. incubation

The infection progress upon room temperature storage was slow and few rots were detected by day 5 of room temperature storage (22-24 °C). The second rot assessment was therefore done after 8 days of room temperature incubation. Less than 50% of fruit was left marketable after 8 days of room temperature storage in non-inoculated (product 1) and inoculated control (product 2), but for different reasons. Fruit in non-inoculated control (product 1) was mainly infected with other rots (~30%), *Botrytis* (~6%) or damaged without visible rots (~6%), while inoculated control (product 2) suffered most from *Monilinia* and other rot infections (~20% each) or damaged without visible rots (~10%). This indicated that: a) the orchard where the fruit was sourced had a latent infections other than *Monilinia*; b) inoculation worked well in increasing brown rot incidence and c) *Monilinia* infections in inoculated control (product 2) most likely masked fruit otherwise infected with other rots.

Notably, even after 8 days of room temperature incubation of highly infected fruit the two fungicide products (product 3 and 4) kept total rots below 20-25% with over 75-80% of healthy marketable fruit. Product 3 controlled *Monilinia*, *Botrytis* and *Penicillium* but not the other rots, while product 4 controlled *Monilinia* and other rots very well, *Penicillium* to much lesser extent and did not control *Botrytis*. The post-harvest dipping of the fruit in fungicide products 3 and 4 did not result in significant levels of residual active ingredient on the fruit. In fact pesticide residue levels for all detected active ingredients were at least 3X below EU and GB MRL levels. Both products are therefore recommended for use as post-harvest control of cherry rots. The optimal choice of product will depend on the type of rot expected to be the main risk based on orchard observations.

All biocontrol products (product 5-8) significantly increased percent of healthy fruit by ~15% in comparison to non-inoculated (product 1) and inoculated control (product 2). The biocontrol products however, did not reduce overall rots to the levels of fungicide

products 3 and 4. Biocontrol products all significantly reduced *Monilinia* infections to varying degrees but were less effective against other rots. As observed in fungicide products, biocontrol products performed differently depending on the type of rot assessed. Product 5 only reduced *Monilinia* infections and the rate of asymptomatic unmarketable fruit (other symptoms) but not *Botrytis*, *Penicillium* or other rots. An interesting example is *Bacillus* based product 6 which maintained the highest amount of healthy fruit of all biocontrol products (~65% at day 8), strongly decreased infections due to *Monilinia*, *Botrytis*, other rots and other symptoms, but significantly increased infections due to *Penicillium*. Residual nutrients supplied by dipping the fruit in the product solution in conjunction with lower susceptibility of *Penicillium* to specific biocontrol effects of *Bacillus* strain present in the product may explain this result.

Similarly to product 6, *Trichoderma* based product 7 significantly increased other rots (*Cladosporium*, *Mucor*, *Trichoderma*, etc) and also rots due to *Botrytis* and *Penicillium*. It did however reduce *Monilinia* infections by ca. 50% from 20% to 10%. Both product 6 and 7 would be therefore most appropriate to use for treatment of fruit with high risk of *Monilinia* and low risk of other rots especially *Penicillium*.

Aureobasidium based biocontrol product 8 was on par with biocontrol product 6 in terms of maintaining ~65% of healthy fruit by day 8, was one of the best products against *Botrytis*, other rots and other symptoms, but was one of the least effective products against *Monilinia* and did not control *Penicillium* at all. This product would therefore be applicable in situations with high risk of *Botrytis* and other rots but not in situations with high *Monilinia* or *Penicillium* risk. More research is needed to ascertain pairwise biocontrol mechanisms (or the lack of them) between pathogens and biocontrol products in commercial condition to understand why some biocontrol products seem to work better against specific pathogens.

This study used high natural inoculum with additional *Monilinia* inoculation. Biocontrol products may work better in lower disease pressure scenario. We thus recommend all biocontrol products for EAMU to control post-harvest fruit rot in cherry.

The only product that did not have any positive effects on the percent of healthy fruit was sterilant product 9. It slightly but significantly reduced *Monilinia* and *Botrytis* infection but increased infections due to *Penicillium* and other rots. Fungal spores and especially latent infection in the fruit may be protected from short lived effects of the sterilant.

For most accurate results on interactions between products and different post-harvest rots we would need to test products on completely clean fruit infected with single rots and then treated with products post-harvest or ideally in orchard conditions. Producing fruit with no infection may only be possible with more fungicide applications than is commercially relevant, resulting in exceeded MRLs and pesticide residuals potentially affecting the outcome of the study.

Table 4: Summary of product effectiveness against different post harvest rots measured in the study. Very effective products reduced post harvest rot by more than

75% (compared to inoculated control); moderately effective products reduced by 50-75% and slightly effective products reduce post harvest rots by 10-50%.

Prod. No.	Product Type	AHDB code	Brown rot (<i>Monilinia spp.</i>)	Grey mould (<i>Botrytis cinerea</i>)	Blue mould (<i>Penicillium spp.</i>)	Other moulds (<i>Mucor, Cladosporium, Alternaria, Fusarium</i>)
3	Fungicide	AHDB 9704	Very effective	Very effective	Very effective	Not effective
4	Fungicide	AHDB 9816	Very effective	Not effective	Slightly effective	Moderately effective
5	Biocontrol	AHDB 9791	Slightly effective	Not effective	Not effective	Not effective
6	Biocontrol	AHDB 9936	Moderately effective	Very effective	Not effective	Slightly effective
7	Biocontrol	AHDB 9788	Moderately effective	Not effective	Not effective	Not effective
8	Biocontrol	AHDB 9758	Slightly effective	Very effective	Not effective	Slightly effective
9	Sterilant	AHDB 9703	Slightly effective	Very effective	Not effective	Not effective

Conclusions

The first conclusion is that low temperature storage (max +2°C) in modified atmosphere bags can almost completely stop cherry post-harvest rot development during 18 days of storage even in the scenario of very high inoculum levels used in this study. The level of unmarketable fruit developed during storage was below ~5% in untreated and below ~3% in treated fruit.

Secondly, all but the sterilant product significantly improved the percent of marketable fruit after 8 days of room temperature incubation compared to controls.

Overall the best products for protection of cherry against post-harvest rots were fungicide products 3 and 4. The products managed to keep over 75% of fruit healthy even after 8 days of room temperature incubation. Dipping of the fruit in the fungicide product solutions did not exceed any pesticide residue limits (MRLs) and are therefore recommended for registration/EAMU for postharvest cherry protection.

Similarly, all biocontrol products significantly increased percent of healthy fruit and reduced *Monilinia* rot, but to a lesser degree than fungicides. The best biocontrol products were product 6 and 8 followed by 5 and 7.

Importantly, the efficacy biocontrol products and to lesser degree fungicides differed depending on the specific rot in question (Table 4). It is therefore important to assess the risk of different rots in the orchard to pick the product that is most appropriate for use in specific circumstances.

Acknowledgements

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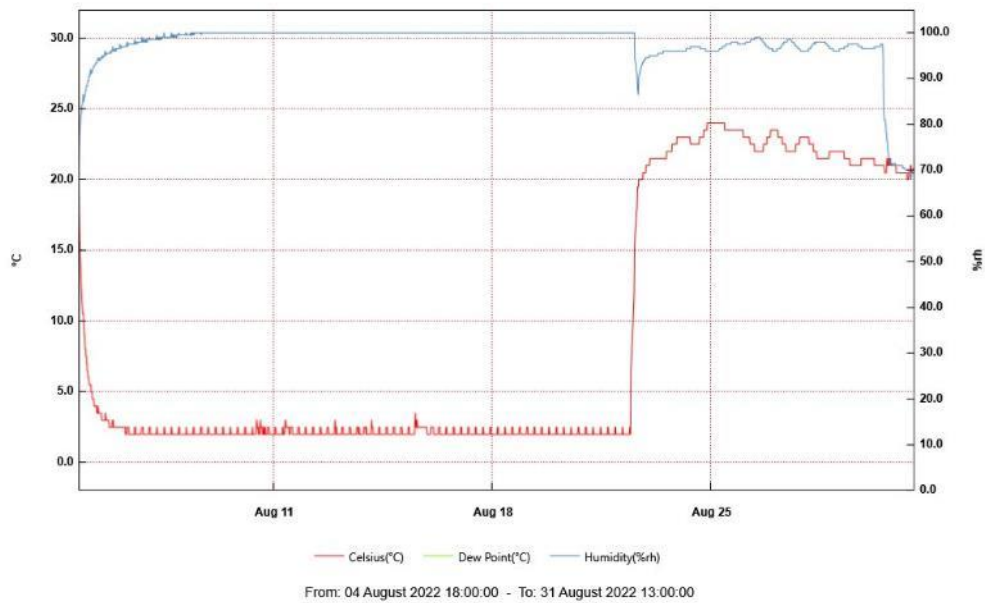
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Appendix

Temperature and humidity during the experiment



Supp figure 1: Temperature and humidity data collected in modified the atmosphere bag next to cherry fruit over the duration of the trial.

Trial Photographs



Supp figure 1. Example of assessment (treatment 5, block 5) at day 8. The fruit was separated into healthy (A), *Monilinia* infected (B), *Botrytis* infected (C), *Penicillium* infected (D), other symptoms/damage (E) and other rots including multiple rots (F).



Supp figure 2: Close up examples of *Monilinia* (left), *Botrytis* (middle) and *Penicillium* (right) infected fruit.

Means and standard errors

Supp table 1. Mean percent of fruit in each category and standard errors (SE) per block and assessment based on raw data.

Assessment	Block	Healthy		<i>Monilinia</i>		<i>Botrytis</i>		<i>Penicillium</i>		Other moulds		Other symptoms	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Day 0	1	96.74	0.53	0	0	0.03	0.03	0	0	2.95	0.51	0.28	0.11
	2	97.45	0.39	0	0	0	0	0	0	2.39	0.42	0.16	0.06
	3	96.93	0.47	0	0	0.11	0.11	0	0	2.79	0.49	0.17	0.09
	4	97.7	0.54	0	0	0.09	0.09	0	0	1.39	0.62	0.81	0.36
	5	96.95	0.74	0	0	0	0	0	0	3.01	0.75	0.04	0.04
Day 8	1	61.45	6.22	12.08	4.04	0.41	0.21	3.33	1.12	20.96	4.27	5.1	2.48
	2	63.59	4.45	10.37	2.86	0.82	0.59	4.75	1.25	19.35	3.08	3.69	1.22
	3	64.6	3.93	10.65	3.7	0.44	0.21	4.83	1.5	19.39	2.37	3.22	0.86
	4	59.02	6.22	9.66	3.83	4.14	3.35	4.69	1.04	21.29	3.48	3.56	1.04
	5	63.39	4.22	3.76	0.9	1.29	0.75	4.11	0.97	25.74	3.84	4.81	1.54

Supp table 2. Mean percent of fruit in each category and standard error (SE) per product and assessment based on raw data.

Assessment	Product	Healthy		Monilinia		Botrytis		Penicillium		Other moulds		Other symptoms	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Day 0	1	95.45	0.96	0	0	0	0	0	0	4.15	1.11	0.4	0.27
	2	97.33	0.83	0	0	0.06	0.06	0	0	2.61	0.78	0	0
	3	97.42	0.87	0	0	0.16	0.16	0	0	1.89	1.07	0.53	0.31
	4	97.68	0.37	0	0	0	0	0	0	2.09	0.26	0.23	0.14
	5	97.61	0.41	0	0	0.2	0.2	0	0	1.87	0.58	0.32	0.25
	6	98.01	1.03	0	0	0	0	0	0	1.79	0.91	0.2	0.13
	7	96.12	0.56	0	0	0	0	0	0	3.81	0.57	0.07	0.07
	8	97.77	0.36	0	0	0	0	0	0	2.23	0.36	0	0
	9	96.99	0.26	0	0	0	0	0	0	2.12	0.62	0.89	0.53
Day 8	1	51.09	4.41	0.47	0.47	8.05	5.69	3.23	0.65	35.59	5.01	6.21	1.83
	2	47.37	4.94	20.13	6.76	0.75	0.46	4.05	0.26	19.93	5.12	10.49	3.63
	3	79.26	4.71	0.71	0.44	0.16	0.16	0.35	0.15	20.74	5.27	1.37	0.37
	4	83.92	2.83	2.86	0.97	1.15	0.97	2.63	0.61	11.24	2.27	0.57	0.46
	5	60.86	1.34	13.34	2.44	1.1	0.74	3.04	1.04	20.78	1.4	3.32	0.73
	6	67.03	1.78	5.88	2.41	0.09	0.09	9.28	2.08	17.44	1.92	2.32	0.64
	7	58.69	3.74	9.24	2.21	1.34	1.18	7.63	1.42	22.98	2.72	4.06	1.59
	8	66.23	4.13	15.64	5.77	0	0	3.36	0.65	15.04	2.43	1.99	0.74
	9	47.21	4.73	15.45	3.29	0.14	0.14	5.53	1.62	28.38	4.68	6.35	1.79

Supp table 3. Model estimated mean percent of fruit in each category and standard error (SE). Groups within the same assessment day denoted with different letters are considered statistically significantly different from each other based on Tukey's post-hoc test ($p < 0.05$).

Assessment	Product	Healthy			Monilinia			Botrytis			Penicillium			Other rot			Other symptom		
		mean	SE	group	mean	SE	group	mean	SE	group	mean	SE	group	mean	SE	group	mean	SE	group
Day 0	1	95.48	0.21	A	0.00	0.00	A	0.00	0.00	A	0.00	0.00	A	4.02	0.20	C	0.26	0.05	BC
	2	97.35	0.16	BC	0.00	0.00	A	0.00	0.03	A	0.00	0.00	A	2.52	0.16	B	0.00	0.00	ABCD
	3	97.44	0.16	BCD	0.00	0.00	A	0.00	0.09	A	0.00	0.00	A	1.82	0.13	A	0.34	0.05	CD
	4	97.71	0.15	BCD	0.00	0.00	A	0.00	0.00	A	0.00	0.00	A	2.02	0.14	AB	0.14	0.03	AB
	5	97.63	0.15	BCD	0.00	0.00	A	0.00	0.11	A	0.00	0.00	A	1.81	0.13	A	0.21	0.04	BC
	6	98.02	0.14	D	0.00	0.00	A	0.00	0.00	A	0.00	0.00	A	1.73	0.13	A	0.13	0.03	AB
	7	96.15	0.19	A	0.00	0.00	A	0.00	0.00	A	0.00	0.00	A	3.69	0.19	C	0.05	0.02	A
	8	97.78	0.15	CD	0.00	0.00	A	0.00	0.00	A	0.00	0.00	A	2.16	0.15	AB	0.00	0.00	ABCD
	9	97.02	0.17	B	0.00	0.00	A	0.00	0.00	A	0.00	0.00	A	2.04	0.14	AB	0.58	0.08	D
Day 8	1	48.79	0.50	B	0.41	0.06	A	5.47	0.24	D	3.06	0.17	B	33.93	0.47	G	5.85	0.23	E
	2	46.10	0.50	A	18.54	0.39	G	0.47	0.06	B	3.90	0.19	C	19.36	0.39	D	10.08	0.30	F
	3	77.20	0.42	F	0.65	0.08	A	0.10	0.03	A	0.34	0.06	A	20.17	0.40	D	1.32	0.11	B
	4	81.99	0.39	G	2.57	0.15	B	0.74	0.07	BC	2.55	0.16	B	10.89	0.31	A	0.54	0.07	A
	5	59.39	0.49	D	12.29	0.33	E	0.69	0.07	BC	2.95	0.17	B	20.16	0.40	D	3.21	0.18	D
	6	65.74	0.48	E	5.32	0.22	C	0.06	0.02	A	9.03	0.29	F	17.02	0.38	C	2.24	0.15	C
	7	56.52	0.49	C	8.29	0.27	D	0.83	0.08	C	7.26	0.26	E	22.01	0.41	E	3.85	0.19	D
	8	64.85	0.48	E	14.32	0.35	F	0.00	0.00	ABCD	3.25	0.18	BC	14.68	0.36	B	1.93	0.14	C
	9	45.77	0.50	A	14.12	0.35	F	0.08	0.02	A	5.33	0.22	D	27.49	0.45	F	6.09	0.24	E

Raw data

Assessment	Block	Plot	Treatment no	Healthy (g)	Monilinia	Botrytis (g)	Penicillium	Other mo	Other sym	Total (g)
day 0	1	1-1	1	1855	0	0	0	67	12	1934
day 0	1	1-2	2	1881	0	6	0	100	0	1987
day 0	1	1-3	3	1923	0	0	0	8	11	1942
day 0	1	1-4	4	1844	0	0	0	49	13	1906
day 0	1	1-5	5	1866	0	0	0	48	0	1914
day 0	1	1-6	6	1873	0	0	0	98	13	1984
day 0	1	1-7	7	1914	0	0	0	46	0	1960
day 0	1	1-8	8	1876	0	0	0	29	0	1905
day 0	1	1-9	9	1848	0	0	0	72	0	1920
day 0	2	1-2	1	1888	0	0	0	81	0	1969
day 0	2	2-2	2	1945	0	0	0	63	0	2008
day 0	2	2-2	3	1926	0	0	0	6	7	1939
day 0	2	2-4	4	1967	0	0	0	24	0	1991
day 0	2	2-5	5	1950	0	0	0	27	6	1983
day 0	2	2-6	6	1868	0	0	0	41	7	1916
day 0	2	2-7	7	1893	0	0	0	73	0	1966
day 0	2	2-8	8	1882	0	0	0	52	0	1934
day 0	2	2-9	9	1914	0	0	0	56	8	1978
day 0	3	1-3	1	1869	0	0	0	63	0	1932
day 0	3	2-3	2	1942	0	0	0	59	0	2001
day 0	3	3-3	3	1819	0	0	0	106	0	1925
day 0	3	4-3	4	1944	0	0	0	49	9	2002
day 0	3	5-3	5	1941	0	20	0	41	0	2002
day 0	3	6-3	6	1931	0	0	0	0	0	1931
day 0	3	7-3	7	1917	0	0	0	64	7	1988
day 0	3	8-3	8	1880	0	0	0	67	0	1947
day 0	3	9-3	9	1928	0	0	0	44	14	1986
day 0	4	1-4	1	1926	0	0	0	33	27	1986
day 0	4	2-4	2	1962	0	0	0	28	0	1990
day 0	4	3-4	3	1939	0	16	0	0	34	1989
day 0	4	4-4	4	1927	0	0	0	35	0	1962
day 0	4	5-4	5	1924	0	0	0	0	25	1949
day 0	4	6-4	6	1995	0	0	0	0	0	1995
day 0	4	7-4	7	1880	0	0	0	116	0	1996
day 0	4	8-4	8	1946	0	0	0	37	0	1983
day 0	4	9-4	9	1924	0	0	0	0	59	1983
day 0	5	1-5	1	1808	0	0	0	163	0	1971
day 0	5	2-5	2	1971	0	0	0	10	0	1981
day 0	5	3-5	3	1924	0	0	0	64	0	1988
day 0	5	4-5	4	1949	0	0	0	49	0	1998
day 0	5	5-5	5	1904	0	0	0	68	0	1972
day 0	5	6-5	6	1913	0	0	0	36	0	1949
day 0	5	7-5	7	1878	0	0	0	77	0	1955
day 0	5	8-5	8	1922	0	0	0	32	0	1954
day 0	5	9-5	9	1947	0	0	0	36	7	1990
day 8	1	1-1	1	1128	0	11	23	722	14	1898
day 8	1	1-2	2	630	93	32	80	653	464	1952
day 8	1	1-3	3	1756	8	0	14	130	0	1908
day 8	1	1-4	4	1626	99	0	39	83	32	1879
day 8	1	1-5	5	1238	187	9	46	386	27	1893
day 8	1	1-6	6	1207	291	0	42	305	54	1899
day 8	1	1-7	7	895	253	0	227	380	163	1918
day 8	1	1-8	8	972	719	0	70	108	7	1876
day 8	1	1-9	9	1049	408	13	32	319	76	1897
day 8	2	1-2	1	1050	0	47	46	720	89	1952
day 8	2	2-2	2	934	466	0	67	379	142	1988
day 8	2	3-2	3	1663	0	0	0	247	14	1924
day 8	2	4-2	4	1554	87	99	93	146	0	1979
day 8	2	5-2	5	1143	386	0	55	286	94	1964
day 8	2	6-2	6	1230	78	0	251	307	29	1895
day 8	2	7-2	7	1280	274	0	128	248	16	1946
day 8	2	8-2	8	1338	243	0	72	242	29	1924
day 8	2	9-2	9	942	294	0	116	396	210	1958
day 8	3	1-3	1	1160	45	12	67	512	120	1916
day 8	3	2-3	2	938	609	35	97	148	140	1967
day 8	3	3-3	3	1435	0	0	11	422	42	1910
day 8	3	4-3	4	1459	62	0	61	326	0	1908
day 8	3	5-3	5	1127	337	0	0	420	34	1918
day 8	3	6-3	6	1402	34	9	257	212	0	1914
day 8	3	7-3	7	1300	108	0	158	293	103	1962
day 8	3	8-3	8	1470	197	0	19	172	68	1926
day 8	3	9-3	9	920	473	0	173	366	25	1957
day 8	4	1-4	1	827	0	581	85	325	92	1910
day 8	4	2-4	2	896	720	0	73	191	50	1930
day 8	4	3-4	3	1532	47	0	9	367	11	1966
day 8	4	4-4	4	1774	0	0	23	133	0	1930
day 8	4	5-4	5	1147	256	0	68	404	50	1925
day 8	4	6-4	6	1273	70	0	148	417	60	1968
day 8	4	7-4	7	1045	207	117	166	402	0	1937
day 8	4	8-4	8	1242	212	0	67	354	77	1952
day 8	4	9-4	9	578	172	0	180	878	136	1944
day 8	5	1-5	1	739	0	120	89	734	243	1925
day 8	5	2-5	2	1240	82	0	79	321	231	1953
day 8	5	3-5	3	1275	15	0	0	664	14	1968
day 8	5	4-5	4	1698	27	15	39	192	0	1971
day 8	5	5-5	5	1211	122	77	125	321	86	1942
day 8	5	6-5	6	1316	89	0	191	258	60	1914
day 8	5	7-5	7	1175	53	13	60	527	104	1932
day 8	5	8-5	8	1348	119	0	95	357	11	1930
day 8	5	9-5	9	1096	151	0	38	593	83	1961

Sequencing data

>GAR915_40739152_40739152_Monilinia_Block1A_Fwd

TAAGAACCTCCCAACCCTTTGTGTATCATTACTTTGTGCTTTGGCGAGCTGCCTTCGGGCCTTGCACGCTCGCC
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Pesticide residue analysis data

Product 2, Inoculated, untreated control



Summary Report

Sample Number	22-001735 0001 000	Date Received	28/10/2022
Customer	NIAB EMR New Road, East Malling Kent ME19 6BJ	Testing Completed	11/11/2022
Contact	Dr A Berrie	Issue Date	11/11/2022
Commodity	Cherries	Report Number	22003573-000
Country of Origin	Unknown		

Customer Reference

Customer Reference T2 / PO: 80140

Residues Detected	Results	Units	RL	EU MRL	GB MRL
Acetamiprid	0.014	mg/kg	0.01	EU 1.5 mg/kg	GB 1.5 mg/kg
Cyantraniliprol	0.017	mg/kg	0.01	EU 6.0 mg/kg	GB 6.0 mg/kg
Fenhexamid	0.027	mg/kg	0.01	EU 7.0 mg/kg	GB 7.0 mg/kg

Notes

RL - Reporting Limits
Tests marked * on this report are not included in the UKAS Accreditation Schedule for this laboratory
Tests marked # on this report are Sub-contracted
Tests marked + on this report indicate not all quality control requirements were met for this residue; these results are therefore considered to be unaccredited
Tests marked F^B on this report are UKAS accredited under our Flexible Scope.
Not Detected implies compounds are not found above the reporting limit
The results as reported, relate only to the item(s) submitted for testing
Test reports shall not be reproduced without the written permission of the laboratory
Measurement of uncertainty is not included in the calculations for results and can be provided on request.
Results in Red highlight MRL exceedances.
Method Ref: QTSM001 (MR1), QTSM010 (MR2), QTSM015 (MR3), standard procedure.
When both EU and GB MRL's are present on the report, the EU MRL will be used to perform the ARID calculation.

Signed on behalf of QTS:

Steven Mann

Position: Laboratory Manager

Tentamus QTS Analytical Ltd, Building 170, Abbott Drive, Kent Science Park, Sittingbourne, Kent, ME98AZ
Tel: 01795 411810

Sample Number 22-001735 0001 000

Opinions and interpretations are not included in the scope of accreditation
The data reported herein is representative of the samples supplied
QTS accepts no liability regarding the use of this information
This report supersedes any previous report

Page 1 of 7



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Tentamus QTS Analytical Ltd

Summary Report

Evaluation of contribution of MRL exceedance to the exhaustion of the acute reference dose (ARfD)

QTS SAMPLE No. 22-001735 0001/Standard

Substance	Content [mg/kg]	MRL ^[1] [mg/kg]	EH MRL [%]	BW ^[2] [kg]	LP ^[2] [g]	VF ^[2]	Intake [mg/kg BW]	ARfD ^[3] [mg/kg BW]	EH ARfD [%]	Comments
Fenhexamid	0.027	7.00	0.4	22.0	269.0	1	0.00033	n.d.		
Acetamiprid	0.014	1.50	0.9	22.0	269.0	1	0.00017	0.02	0.67	
Cyantraniliprol	0.017	6.00	0.3	22.0	269.0	1	0.00020	n.d.		
	Sum		1.6					Sum	0.67	

Substance	Infant		Toddler		4-6yr olds		Adult		*VF, BW and LP for NESTI calculation taken from [3]
	Nesti*	EH ARfD [%]	Nesti*	EH ARfD [%]	Nesti*	EH ARfD [%]	Nesti*	EH ARfD [%]	
Fenhexamid	0.00007	n.d.	0.00012	n.d.	0.00014	n.d.	0.00005	n.d.	
Acetamiprid	0.00004	0.15	0.00006	0.24	0.00007	0.30	0.00003	0.10	
Cyantraniliprol	0.00005	n.d.	0.00007	n.d.	0.00009	n.d.	0.00003	n.d.	

LP = Large Portion
 VF = Variability Factor
 BW = Body Weight for [2] (average weight of children, based on data for Denmark)
 EH = Exhaustion
 MRL = Maximum Residue Level
 Sub. = Substance
 n.d. = No data

Data sourced from: [1] EU Pesticide Database or current EFSA risk assessments, [2] EFSA PRIMo Database, [3] CRD NESTI model.
 Disclaimer: ARfD reports are not UKAS accredited. Clients are advised to check with the relevant authorities as to marketability of produce.
 Tentamus QTS cannot accept any liability for mistakes arising from the inaccurate use of this spreadsheet.

Signed on behalf of QTS:

Steven Mann

Position: Laboratory Manager

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Sample Number 22-001735 0001 000

Opinions and interpretations are not included in the scope of accreditation
 The data reported herein is representative of the samples supplied
 QTS accepts no liability regarding the use of this information
 This report supersedes any previous report

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ORETO certificate



Certificate of

Official Recognition of Efficacy Testing Facilities or Organisations in the United Kingdom

This certifies that

NIAB EMR

complies with the minimum standards laid down in
Regulation (EC) 1107/2009 for efficacy testing.

The above Facility/Organisation has been officially
recognised as being competent to carry out efficacy trials/tests
in the United Kingdom in the following categories:

**Agriculture/Horticulture
Biologicals and Semiochemicals
Stored Crops**

Date of issue: 12 July 2018
Effective date: 1 January 2018
Expiry date: 31 December 2022

Signature

Authorised signatory

Certification Number

ORETO 411



Chemicals Regulation Division



Department of

**Agriculture and
Rural Development**