SCEPTREPLUS

Trial Report

Trial code:	SP 68
Title:	New strategies to control apple canker
Crop:	Apple
Target:	European apple canker disease, *Neonectria ditissima*
Lead researcher:	Dr Matevz Papp-Rupar
Organisation:	NIAB EMR (East Malling Research)
Period:	1st April 2020- 31st Dec 2021
Report date:	31.12.2021
Report author:	Dr Matevz Papp-Rupar
ORETO Number: (certificate should be attached)	Certificate No. 411

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: 31.12.2021

Author's signature:
Matevz Papp-Rupar

Grower Summary

Introduction

Apple canker, caused by the fungus *Neonectria ditissima* (Tul. & C. Tul.) (Samuels & Rossman), is one of the most important diseases of apple. The fungus infects trees in the orchard through wounds causing cankers and die back of shoots. Apple canker can be particularly damaging in young orchards where up to 20% of trees can be lost per year in the first few years of orchard establishment because of trunk cankers. *N. ditissima* also causes fruit rot that can result in losses as high as 10% or more of stored fruit. Fungicides effective against canker, particularly for use at leaf fall, are limited. Moreover, since June 2019, copper oxychloride products most commonly used for suppression of infection at bud burst and leaf fall are no longer available under emergency authorisation in UK. This project evaluated several new fungicides, biocontrol agents (BCAs) and alternative chemicals, for their efficacy in controlling *N. ditissima* canker at leaf fall. Products were chosen after consultation with growers, agronomists, agro-chemical companies, other industry stakeholders and SCEPTREplus consortium members.

Methods

Products were first screened in laboratory assays for their ability to reduce *N. ditissima* spore germination in water suspensions and mycelium growth on agar plates. A subset of the top eight most effective products that are likely to get approval in the near future were selected for a randomised orchard trial (6 blocks, 5 trees per plot). The products were sprayed on approximately 10-year-old Braeburn trees during the leaf fall of 2020 (at 0-90% leaf fall) using a motorised knapsack sprayer. Depending on the product, we applied between two and four applications to achieve maximal registered dose for each product. Leaf scars and pruning wounds were created on the trees a few hours prior to the first protectant spray application. Following application of the products, a subset of leaf scars on each tree were then inoculated with a mix of *N. ditissima* conidia. The rest of the leaf scars and pruning wounds were left to get infected naturally. The trial orchard had alternating rows of Gala trees with several cankers per tree serving as natural inoculum. Canker frequency on the test trees was assessed in June 2021.

Results

In laboratory assays product numbers 1 (AHDB9891) and 12 (AHDB9792) reduced *N. ditissima* spore germination to ~12% and ~36% of the water control, respectively. Products 3 (AHDB9936), 6 (AHDB9794), and 11 (AHDB9795) inhibited germination completely. All products significantly reduced *N. ditissima* mycelium growth on the agar plates with the exception of products 4 and 13. The products that reduced the growth of *N. ditissima* mycelium to below 60% of water control were selected for trials in the orchard. Only product 6 (AHDB9794) significantly reduced canker incidence in the orchard trial.

Conclusions

Fungicide product 6 (AHDB9794) was the most effective control of *N. ditissima* infecting leaf scars at leaf fall. Fungicide product 7 (AHDB9862) and surfactant product 12 (AHDB9792) showed slight, but not significant control and may offer commercially relevant control when applied as a mixture. Biological control agents applied 4x during the leaf fall provided very little apple canker control in the field. Product 17 (AHDB9787) and 3 (AHDB9936) provided slight, but not significant control in artificially inoculated leaf scars and product 2 (AHDB9791) in natural infections only.

Take home message
The trials have identified fungicide product 6 (AHDB9794) applied twice during the leaf fall to be an effective control of *N. ditissima* infecting leaf scars in high and low disease pressure scenarios.

Objectives

1) To screen fourteen products in *in-vitro* lab assays for activity against *N. ditissima* and select the top eight for evaluation in the orchard trial.
2) Test pruning wound and leaf scar protection efficacy of the top eight products at leaf fall against *N. ditissima* in the orchard trial.

Methods

UK regulatory guidelines were followed, but EPPO guidelines took precedence. The following EPPO guidelines were followed:

Relevant EPPO guideline(s)		Variation from EPPO
PP1/152(4)	Design and analysis of efficacy evaluation trials	None
PP1/181(4)	Conduct and reporting of efficacy evaluation trials including good experimental practice	None
PP1/239(2)	Dose expression for plant protection products (PPPs)	None
PP1/223(2)	Introduction to the efficacy evaluation of plant protection products	None

Test site

Item	Details
Location address	Pathology laboratory, Middle Park apple orchard, plot 196 NIAB EMR, New Road, East Malling, Kent, ME19 6BJ
Crop	Apple
Cultivar	Braeburn

Trial design

Item	Details
Trial design:	Both orchard (6 blocks) and laboratory (3 blocks) trials were done in randomised complete block design.
Number of replicates:	Three replicates in laboratory trial and 6 in the orchard trial.

Treatment list

Table 1. List of treatments used in the study

Prod. no.	Code	Type	Rate (based on 500 l/ha spray volume)	Reason for inclusion
1	AHDB9891	Biocontrol agent	5 g/L	Authorised strain
2	AHDB9791	Biocontrol agent	0.4 g/l	Authorised strain
3	AHDB9936	Biocontrol agent	20 ml/L	Authorised strain
4	AHDB9796	Biocontrol agent	3 g/L	Authorised strain
5	AHDB9788	Biocontrol agent	1.5 g/L	Registered for trunk disease on grapes.
6	AHDB9794	Fungicide	3.2 ml/L	Proposed by SCEPTRE-industry meetings
7	AHDB9862	Fungicide	3 ml/L	Company to support expansion into fruit market
8	AHDB9808	Fungicide	9 ml/L	Potential replacement for copper oxychloride
9	AHDB9926	Fungicide	1.8 ml/L	Proposed by SCEPTRE committee
10	AHDB9789	Fungicide	20 ml/L	Proposed by SCEPTRE-industry meetings
11	AHDB9795	Fungicide	13.5 g/L	Proposed by SCEPTRE committee
12	AHDB9792	Other	3.9 ml/L	Proposed by SCEPTRE committee
13	AHDB9793	Other	8 ml/L	Tested in IR-4, coming to UK market soon
14	AHDB9790	Other	5 ml/L	Proposed by SCEPTRE-industry meetings
15	Water	Negative control	/	/
16	AHDB9787	Positive control	1.2 ml/L	Previously registered for control of canker

Spore germination inhibition assay

Water suspensions of the test products were mixed with *N. ditissima* spore suspension (at 2 x 10^4 macroconidia per mL) on glass slides. Every test droplet consisted of 10µl of spore suspension and 10µl of product suspension at twice the recommended concentration to account for dilution due to mixing with spores. Slides were incubated in high humidity boxes at room temperature for 48h after which trypan-blue was used to fix and stain the test droplets. Microscopy was then used to assess germination. Spores with germ tubes longer than the width of the spore were considered germinated. Between 30 and 100 spores were inspected in every test droplet.
Three dilutions of each product were tested; maximum application concentration at 500 L/ha according to product labels (Table 1), 5x and 25x dilution of the maximum application concentration (Figure 1). Three replicate test droplets per product per

concentration were assessed. Water was used as a negative control and a tebuconazole based product (treatment 16) as a standard.

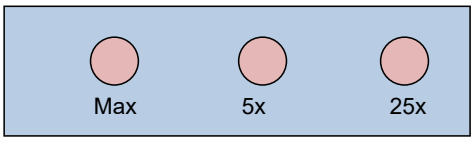


Figure 1. Spore germination assay slide diagram. Three slides per product were prepared.

Mycelium growth inhibition assay

N. ditissima cultures were grown at room temperature on potato dextrose agar (PDA) plates (9cm in diameter) for six days until mycelium reached approximately 3cm in diameter. Droplets of test product suspensions (20 µl) were then applied to the agar plates. Two different assay formats were used to assess products. Droplets with biological control products were applied at 2 cm from the edge of mycelium (Figure 2) and the growth of mycelium towards the droplets was measured after seven days (Format 1). Droplets with fungicide and other chemicals were applied next to the edge of the mycelial culture (Figure 2) and the growth of mycelium over the product droplet was measured after five days (Format 2). Product droplets were applied at 2x the recommended concentration to account for dilution due to absorption in the agar media. A water control was used on every plate as a negative control and the tebuconazole based product (treatment 16) was used as a standard (Figure 2). Each product at each of the treatment concentrations was tested in triplicate.

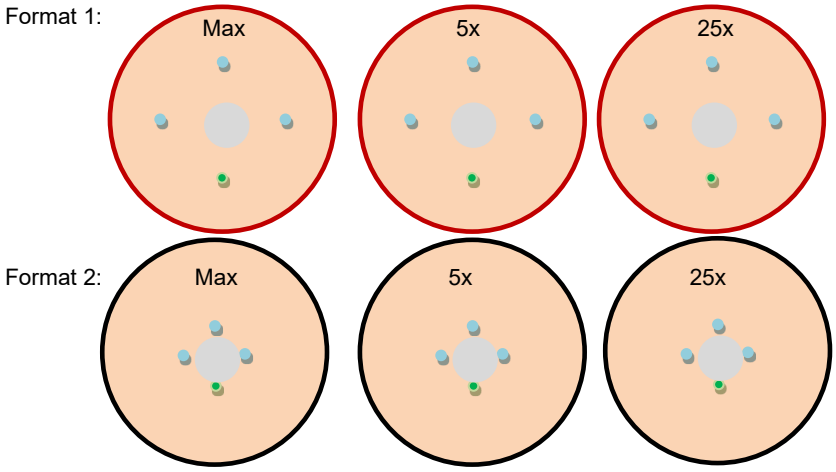


Figure 2. Mycelium growth assay diagram. Water control droplets are depicted in green, treatment droplets in blue and *N. ditissima* mycelium in grey. The format used for biocontrol agents is outlined in red and the format used for fungicides and other chemicals is outlined in black.

Orchard trial

The orchard trial was conducted in an approximately 10-year-old orchard with alternating rows of Gala and Braeburn trees on M9 rootstock, with 1.5 m between trees

and 3.5 m between rows. Gala trees had a very high apple canker incidence and were used as natural inoculum. Treatment application and assessments were done on the Braeburn trees. The trial was conducted in a fully replicated compete block design with 10 treatments (Table 2) in six blocks, one plot per treatment per block. Each plot consisted of five trees of which the middle three trees were used for inoculation and assessments. A minimum of two buffer trees were used between each two neighbouring plots within the same row.

Application schedule

Table 2. Orchard trial, product application schedule. Fields in grey denote the weeks when applications of a product were done. Application rates are listed in Appendix 2. Product 17 was not tested in laboratory trials and was recommended as a replacement for product 5 (AHDB9788) by the SCEPTREplus panel.

Product number (AHDB code)	No. of applications	Product type	Week starting	19/10	26/10	09/11	23/11
			Plant stage		10 % leaf fall	50 % leaf fall	90 % leaf fall
1 AHDB9891	4	Biocontrol agent					
2 AHDB9791	4	Biocontrol agent					
3 AHDB9936	4	Biocontrol agent					
17 AHDB9955	4	Biocontrol agent					
6 AHDB9794	2	Fungicide					
7 AHDB9862	3	Fungicide					
8 AHDB9808	4	Other					
12 AHDB9792	4	Other					
16 AHDB9787	3	Positive control					
15 Water	4	Negative control					

The efficacy of the products to prevent infection of apple leaf scars and pruning wounds was tested at leaf fall. Multiple leaf fall applications of the products were done (Table 2). The number of applications and application rates were based on the product labels (Appendix 2). The maximum dose of each product (per Ha) was applied in two to four applications (Table 2, Appendix 2). Applications of biocontrol products and two non-fungicidal chemicals started one week after harvest to build up the biocontrol population/product concentration on the trees prior to leaf fall. The applications of fungicides started at 10% leaf fall followed by fortnightly applications until 90% leaf fall (Table 2). Treatments were applied as a spray using a motorised knapsack sprayer (Birchmeier) with orange Albuz ART 80 nozzles at 500L/Ha. The only exception was the treatment application on 23/11/2020 when the application volume was reduced to 300 L/Ha due to the limited canopy density at 90% leaf fall. Spray application efficiency was between 97% and 102%.

Inoculation

After the fruit has been harvested (12.10.2020) five healthy shoots were marked on each of the middle three trees per plot. On the morning of 26/10/2020 all leaves were stripped from the marked shoots and the top 3 cm of each shoot was cut off with secateurs to simulate a pruning wound. One pruning wound and between 15-30 leaf scars were created on each shoot. Spray application of products was done on the same day in the afternoon to protect fresh leaf scars and pruning wounds immediately and give the products the highest possibility of suppression of infection. The next day (Appendix 3) four leaf scars per shoot were inoculated with 4µl of a *N. ditissima* spore suspension (9.8 x 10^4 spores/ml). A mix of conidia from three different *N. ditissima* strains was used: Hg199 - 50%, R6/20 - 25%, R7/20 - 25% (spore germination in water was 92.5%). The rest of the leaf scars and the pruning wound on each shoot were left for natural inoculation over the winter. This created three distinct infection scenarios to test the products in:

1) Leaf scars under high disease pressure (inoculated) – approximately 60 per plot
2) Leaf scars under natural inoculation pressure – approximately 200 per plot
3) Pruning wounds under natural inoculation pressure – approximately 15 per plot.

Assessment

The orchard trial was assessed on June 3rd, 2021. Disease incidence in inoculated leaf scars, naturally infected leaf scars and pruning wounds was assessed. Obvious canker lesions were considered the result of successful infection.

Statistical analyses

Statistical analysis of laboratory data was performed in Excel. A T-test was used to compare *N. ditissima* spore germination (%) and mycelial growth (mm) of treatments to negative (water) and positive control (standard). The orchard trial data was analysed using R in R-Studio. The proportion of successful infections in the orchard trial was modelled separately for each of the three infection scenarios (leaf scar artificial infection, leaf scar natural infection, pruning wound natural infection) using logistic regression. If the data was over-dispersed, the model was refitted using the quasi-binomial family with logit link function. Analysis of deviance was used to check for an overall treatment effect. Post-hoc means and contrasts were estimated using the R package ‘emmeans’. Contrasts between the water control and treated plots were controlled for by family-wise error using Dunnett's test.

Results

Laboratory trial results

Spore germination assay

The ability of products to inhibit or reduce germination of *N. ditissima* spores is the most effective way to prevent infection. Spore germination in the water control (treatment 15) was ~93 %. Microscopy images of different germination rates are presented in Appendix 4. For each treatment we calculated the percentage of germination compared to the control (germination in product/germination in control). The results from the assay where spores were exposed to the maximum field rate of the product are presented in Figure 3. Mean germination rates per product at different application rates are in Appendix 5. Remarkably, the standard fungicide at full rate (treatment 16, AHDB9787, tebuconazole) did not significantly reduce germination compared to the control. Inspection under the microscope (Appendix 4) revealed that although the spores germinated, the resulting germ tubes did not grow as long as in water control. Five products at the maximum application rate significantly reduced germination of *N. ditissima* spores (Figure 3). Products 1 (AHDB9891) and 12 (AHDB9792) reduced germination to ~12% and ~36% of the water control, respectively. Products 3 (AHDB9936), 6 (AHDB9794), and 11 (AHDB9795) inhibited germination completely. All the products that significantly reduced *N. ditissima* spore germination rate were selected for trial in the orchard.

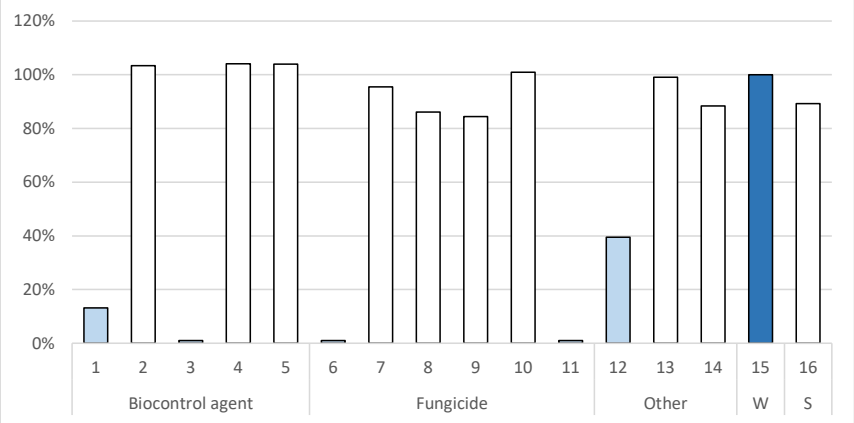


Figure 3. Effect of different crop protection products (applied at maximum rate) on germination of *N. ditissima* spores as a percent of water control (dark blue - W). Treatment 16 was used as standard (S). Products with a significantly lower germination rate than the water control are highlighted in light blue.

Mycelium growth assay

We tested the ability of products to inhibit or reduce *N. ditissima* mycelium growth in two different formats. Biocontrol agents were tested in format 1 and the rest of the products in format 2. The water control and standard were used in both formats. Representative images from both assay formats are presented in Appendix 4. The mycelial growth rate as a percent of the water control was calculated. The results from the assay where *N. ditissima* mycelium was exposed to the maximum field rate of the products are presented in Figure 4. The mean growth rates and statistical test for all different product rates are in Appendix 5. The standard fungicide treatment and the majority of the test products significantly reduced *N. ditissima* mycelium growth (Figure 4). The most efficacious products were 11 (fungicide) and 5 (BCA) reducing mycelial growth down to approximately 5% and 20% of the water control, respectively. Products 1, 3, 6 and 7 all reduced mycelial growth to approximately 30% of the control and the products 2, 8, 9, 10, 12 and 14 reduced the growth to between 50% and 80% of the control. Products no. 4 (AHDB9796) and 13 (AHDB9793) did not significantly reduce mycelial growth. The products that inhibited mycelial growth to below 60% of the water control were selected for the orchard trial.

Commented [CJ1]: Give some indication on amount of inhibition for best / worst products etc

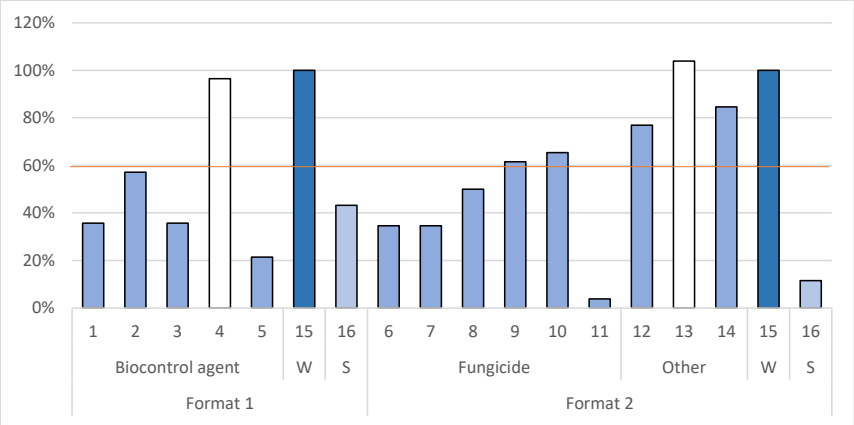


Figure 4. Effect of different crop protection products (applied at maximum rate) on mycelium growth of *N. ditissima* on agar plates as a percent of water control (15, dark blue - W). Treatment 16 was used as standard (S). Products with a significantly lower mycelium growth than the water control are highlighted in light blue. The orange line denotes the growth rate equivalent to 60% of control.

Selection of products for inclusion in the orchard trial

The size of the trial orchard was limited to ten treatments comprising eight test products, the water control and standard. The results of the laboratory trials were discussed with the SCEPTRE panel and the decision on the final list of products used in the orchard trial was made with the panel. Product 5 (AHDB9788) was considered too far away from being approved in the near future and was thus replaced with product 17 (AHDB9955) which is also based on a related biocontrol organism and already approved. Product 11 (AHDB9795) was removed from the orchard trial since its copper based active ingredient is unlikely to get approval in the future. The products selected and associated decisions are listed in Table 3.

Table 3. Combined results from laboratory assays (germination and mycelium growth) with decision whether to include the product in the orchard trial. Numbers in bold denote a significant reduction in comparison to the water control.

Pr. no	Code	Type	Germination (% of water control)	Growth (% of water control)	To orchard trial?
1	AHDB9891	Biocontrol agent	**13%**	**36%**	Yes
2	AHDB9791	Biocontrol agent	103%	**57%**	Yes
3	AHDB9936	Biocontrol agent	**0%**	**36%**	Yes
4	AHDB9796	Biocontrol agent	104%	96%	No
5	AHDB9788	Biocontrol agent	104%	**21%**	No [1]
6	AHDB9794	Fungicide	**0%**	**35%**	Yes
7	AHDB9862	Fungicide	95%	**35%**	Yes
8	AHDB9808	Fungicide	86%	**50%**	Yes
9	AHDB9926	Fungicide	84%	**62%**	No
10	AHDB9789	Fungicide	101%	**65%**	No
11	AHDB9795	Fungicide	**0%**	**4%**	No [2]
12	AHDB9792	Other	**39%**	**77%**	Yes
13	AHDB9793	Other	99%	104%	No
14	AHDB9790	Other	88%	**85%**	No

[1] Product 5 was substituted with product 17 (Table 2) which has a related biological control agent and was already approved in 2020 (requested by SCEPTRE panel member)

[2] Product11 was removed from the orchard trial shortlist because its copper based active ingredient is not likely to get approval.

Orchard trial

For ease of interpretation, we used orchard ID (abbreviated product type followed by product number) (Table 2) when presenting the orchard trial result figures. In the trial, we tested the efficacy of treatments as protectants, i.e., fresh leaf scars and pruning wounds were sprayed within a few hours of creation, followed by additional fortnightly spray applications (Table 2) to simulate a best-case scenario for leaf fall spray. If a product does not control apple canker in this approach, it is unlikely to control canker in commercial practice. Images of the trial are presented in Appendix 4.

Probability of infection in artificially inoculated leaf scars

Infection success in artificially inoculated leaf scars was ~15% (Figure 5, 15-Water control) which is low but not unusual for the investigated pathosystem. Block and treatment both significantly affected the probability of canker symptoms on artificially inoculated leaf scars (Table 4). Very light rain started during the inoculation of blocks 3 and 6 which might explain the block effect.

Table 4. Probability of canker on artificially inoculated leaf scars, ANOVA table of factors. Pr(>Chi) < 0.05 denotes a factor with significant effect.

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL	NA	NA	59	450.2118	NA
Block	5	99.38332	54	350.8285	0.0001656
Treatment	9	147.21298	45	203.6155	0.0000329

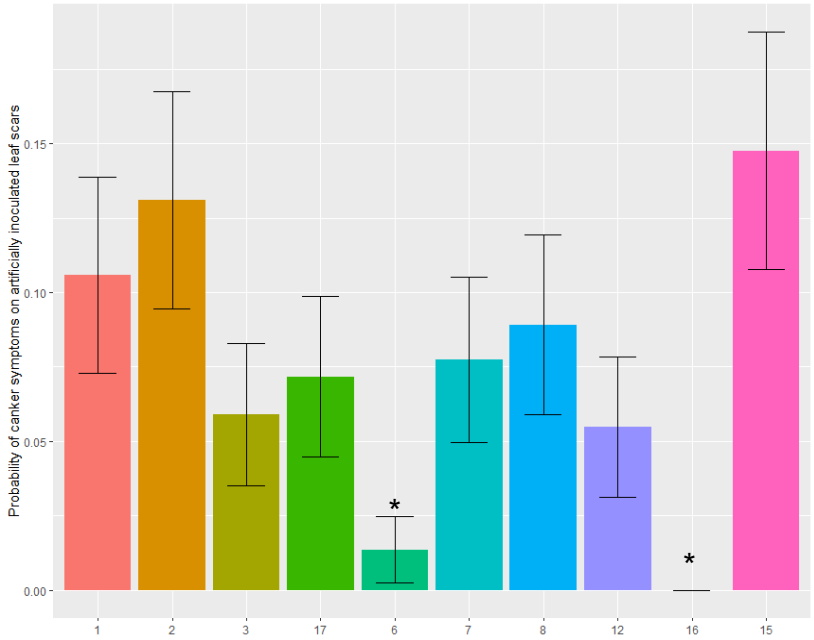


Figure 5. Effect of crop protection products on *N. ditissima* infection rate (probability) on artificially inoculated leaf scars, means +/- SEM. Treatments significantly different from the water control (15) are denoted with *. Treatment 16 was standard fungicide(tebuconazole)

Treatment had a significant effect on the probability of canker symptom expression upon inoculation (Table 4), but only 2 treatments significantly reduced the probability of infection in comparison with the water control; the standard fungicide (16) and product no. 6 (AHDB9794) (Figure 5, Table 5).

Table 5. Effect of crop protection products on *N. ditissima* infection rate (probability) on artificially inoculated leaf scars; statistical comparison of treatments with the water control.

Contrast	p.value
1 - 15	0.9153946
2 - 15	0.9971893
3 - 15	0.2857835
17 - 15	0.4848540
6 - 15	**0.0316981**
7 - 15	0.5649812
8 - 15	0.7437856
12 - 15	0.2493375
16 - 15	NA *

* The p value of the standard versus water contrast cannot be calculated in a binomial model because the standard had no leaf scars with canker lesions. The groups are considered significantly different.

Naturally infected leaf scars

N. ditissima infection success in naturally infected leaf scars was ~3% (Figure 6, Water control) which is expected for the investigated pathosystem. Treatments significantly affected the probability of canker symptoms on naturally infected leaf scars (Table 6). Products 2, 6 and the standard (16) reduced the probability of natural infections by approximately 50%, 66% and 75%, respectively in comparison to the water control (Figure 6). However, none of these differences were not statistically significant (Table 7).

Table 6. Probability of canker on naturally infected leaf scars, ANOVA table of factors. Pr(>Chi) < 0.05 denotes a factor with significant effect.

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL	NA	NA	59	160.64119	NA
Block	5	13.66711	54	146.97408	0.2458288
Treatment	9	53.50315	45	93.47093	0.0019402

Table 7. Effect of crop protection products on *N. ditissima* infection rate (probability) on naturally infected leaf scars; statistical comparison of treatments with the water control.

Contrast	P.value
1 - 15	0.9624626
2 - 15	0.8412627
3 - 15	0.4445012
17 - 15	0.9943944
6 - 15	0.2863312
7 - 15	0.9795391
8 - 15	0.9999965
12 - 15	1.0000000
16 - 15	0.4795417

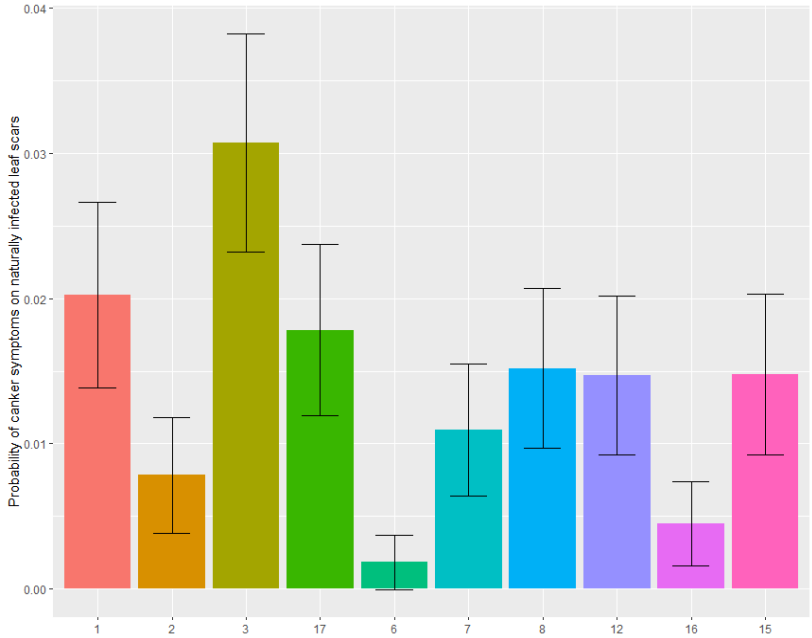


Figure 6. Effect of crop protection products on *N. ditissima* infection rate (probability) of naturally infected leaf scars, means +/- SEM.

Pruning wounds

The mean rate of natural *N. ditissima* infection of pruning wounds was approximately 8% (Figure 7) which is expected. Treatment had no effect on the rate of pruning wound infection (Table 8) and there were no significant differences between any of the treatments including the standard and the water control (data not shown).

Table 8. Probability of canker on artificially inoculated leaf scars, ANOVA table of factors. Pr(>Chi) < 0.05 denotes a factor with significant effect.

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL	NA	NA	59	89.72419	NA
Block	5	9.911611	54	79.81258	0.2245718
Treatment	9	7.241142	45	72.57144	0.8276627

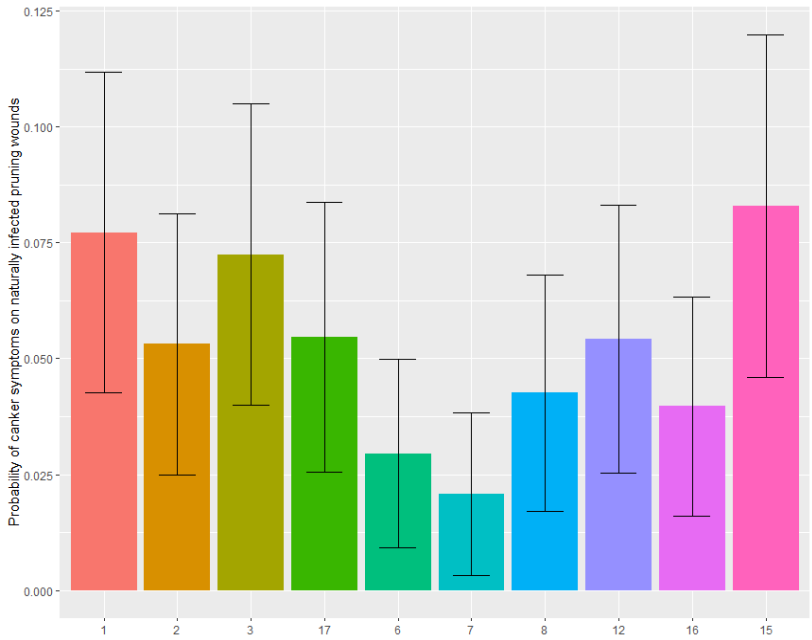


Figure 7. Effect of crop protection products on *N. ditissima* Infection rate (probability) of pruning wounds, means +/- SEM

Discussion

We successfully developed laboratory-based bioassays to screen products for the control of *N. ditissima in-vitro*. We used a germination inhibition assay and mycelium growth inhibition assay to identify 8 out of 14 crop protection products with activity against the pathogen for test in an orchard trial. Here, the efficacy of products to control *N. ditissima* infection of pruning wounds, artificially inoculated and naturally infected leaf scars was investigated with product 6 (AHDB9794) exhibiting significant disease control.

The most frequent critiques of orchard trials are the choice of inoculum concentration/pathogen strain, the choice of inoculation wound and number/timing of product application. In our study we addressed this by using both natural and artificial inoculation on leaf scars and pruning wounds, and also a mix of three pathogen strains to comprehensively assess each product in different disease pressure settings. We used a wound protection approach by applying products immediately after wound exposure, followed by 1-3 subsequent applications up to the maximum registered dose of each product per Ha per season. The spray applications were done with a motorised, air assisted knapsack sprayer to best simulate commercial spray practice. This approach measured the maximum field efficiency of each product.

Four biological control agents were chosen for assessment in orchard trials. *Bacillus spp.* based products 1 (AHDB9891) and 3 (AHDB9936) inhibited both *N. ditissima* spore germination and mycelial growth *in vitro*. *Bacillus spp.* based product 2 (AHDB9791) inhibited only mycelial growth, signifying different modes of action of different BCA strains and possible differences in product formulations. The spore germination inhibition was measured in water within very short period of time (around 48h), and thus likely to be the result of the bioactive compounds present in formulations of products 1 and 3 rather than produced by live BCAs after mixing with *N. ditissima* spores on the slides.

Mycelium growth was reduced by *Trichoderma spp.* based product no. 5 (AHDB9788) but the inhibition was not due to secretion of bioactive compounds but rather through fast growth and competition for space and nutrients (see Appendix 5). Upon request of the SCEPTRE panel members the product no. 5 was replaced by another *Trichoderma spp.* based product no. 17 (AHDB9955) in the orchard trial.

None of the biological control agents significantly reduced *N. ditissima* infection in the orchard trial. Product 3 (AHDB9936) and 17 (AHDB9955) reduced the probability of canker developing on artificially inoculated leaf scars by around 50% and product 2 (AHDB9791) also reduced the probability of cankers on naturally infected leaf scars by around 50%, but these results were too variable for a statistically significant effect. Product 1 (AHDB9891) which inhibited both *N. ditissima* spore germination and mycelium growth in the lab assay was not effective in the field. This is in line with other reports of poor translation of BCA efficacy from the lab into field (Walter et al., 2017). One of the reasons is that *N. ditissima* spores can survive at low temperatures and in a low nutrient environment on apple trees over the winter (Saville & Olivieri, 2019), and require as few as 5 spores to cause a canker (Walter, 2016) while the BCAs require either higher temperatures, more nutrients or both to establish and control canker.

We propose two possible improvements for BCA efficacy in the orchard trials: 1) more frequent applications with a lower rate and higher application volume which would increase the probability of BCA establishment on the wounds; 2) development of

endophytic BCAs that colonise the leaves, petioles and shoots prior to leaf-fall and protect the wounds from within the plant.

Three of the fungicides tested and one other chemical product were included in the orchard trial based on their performance *in vitro*. All fungicide products significantly reduced *N. ditissima* mycelial growth in the laboratory assay but only products 6 (AHDB9794) and 11 (AHDB9795) reduced spore germination as well. None of the fungicides or other chemical products were as effective in reduction of mycelial growth as the standard fungicide, i.e. all products were significantly worse than the standard (Appendix 5). The exception was product 11 which reduced *N. ditissima* mycelium growth to the levels comparable to standard. Interestingly, the standard fungicide product 16 (AHDB9787, tebuconazole) reduced mycelial growth but not spore germination. However spore germ tubes were shorter than in the water control (Appendix 4). SCEPTREplus panel members advised to exclude product 11 from the orchard trial due to its copper based active ingredient as it is unlikely to get approval in the future.

In the orchard trial, product 6 (AHDB9794) and the standard fungicide significantly reduced the probability of canker developing on artificially inoculated leaf scars. They both reduced the probability of canker on naturally infected wounds as well by between 60-80%, but the differences in comparison to the water control were not significant due to the high variability of the natural infections. Product 7 (AHDB9862) and 8 (AHDB9808) reduced canker on all artificially inoculated leaf scars and pruning wounds by around 50% but the reduction in comparison to the water control was not significant. Product 12 (AHDB9792) also slightly, but not significantly reduced canker on artificially infected leaf scars and pruning wounds. Since this product is a wetter/surfactant it could be used in combination with other products to increase the overall efficacy. It could also be used at a higher application frequency (weekly) during the leaf fall to increase the contact time and further improve efficacy.

The reduction of *N. ditissima* mycelial growth observed in most of the chemical products in the laboratory trials was not enough to significantly reduce canker in the field. Only product 6 which reduced both spore germination and mycelial growth in the lab was able to control *N. ditissima* in the field.

Cost and labour availability to apply multiple application at the optimal time together with a low number of effective products are the limiting factors for effective apple canker control in commercial production. To respond to this, we propose that product mixes of surfactants with fungicides or fungicides with BCAs should be investigated in the future.

In consultation with industry representatives, we also identified two BCAs that were not tested in the current trial but have the potential to control apple canker in the future. The first is *Trichoderma spp.* based treatment 5 (AHDB9788), which was effective in our laboratory trials and is being approved for use in the near future. The second is yeast based product 4 (AHDB9796) which did not inhibit *N. ditissima* spore germination or mycelium growth in laboratory assays but has the potential to be an effective control agents due to its predicted high degree of tree colonisation and competition for nutrients with *N. ditissima*.

Conclusions

The trials have identified fungicide product 6 (AHDB9794) applied twice during the leaf fall period to be an effective control method for *N. ditissima* infecting leaf scars in both high and low disease pressure scenarios. Fungicide products 7 (AHDB9862), 8 (AHDB9808) and surfactant product 12 (AHDB9792) showed slight but not significant control and may offer commercially applicable control when mixed together.

Biological control agents applied four times during leaf fall provided very little canker control in the field. Product 17 (AHDB9787) and 3 (AHDB9936) provided slight, but not significant control in artificially inoculated leaf scars and product 2 (AHDB9791) in natural infections only.

References

M Walter. (2016). How many conidia are required for wound infection of apple plants by Neonectria ditissima? *N Z Plant Prot*, *69*, 238–245.

Saville, R., & Olivieri, L. (2019). *Fungal diseases of fruit: apple cankers in Europe* (pp. 59–84). Burleigh Dodds.

Walter, M., Campbell, R. E., Amponsah, N. T., Turner, L., Rainham, D., Kerer, U., & Butler, R. C. (2017). Can biological products control Neonectria ditissima picking wound and leaf scar infections in apples? *New Zealand Plant Protection*, *70*(Swinburne 1975), 63–72. https://doi.org/10.30843/nzpp.2017.70.29

Acknowledgements

Several acknowledgements are due in relation to the successful delivery of this work. We would like to thank advisers from product providing companies for their assistance in choosing appropriate application rates and timing, Greg Deakin for statistical analysis and Tom Passey, Jennifer Kingsnorth, Georgina Fagg, Ben Brough, Hamish McClean, Josh Weaver and Joyce Robinson for technical support with the experiments.

Special thanks goes to Jenifer Kingsnorth and Dr Netsai Mhlanga for proof reading the final report.

We would like to thank the AHDB and all the companies supporting SCEPTREplus for funding this work.

Finally, thank you to the SCEPTREplus panel for their guidance and support with this project during a challenging year.

Appendixes:

Appendix 1: Treatment codes (sensitive details removed for coded products)

Product no.	Producer	Product	Active substance	Code	Type
1				AHDB9891	Biocontrol agent
2				AHDB9791	Biocontrol agent
3				AHDB9936	Biocontrol agent
4				AHDB9796	Biocontrol agent
5				AHDB9788	Biocontrol agent
6				AHDB9794	Fungicide
7				AHDB9862	Fungicide
8				AHDB9808	Fungicide
9				AHDB9926	Fungicide
10				AHDB9789	Fungicide
11				AHDB9795	Fungicide
12				AHDB9792	Other
13				AHDB9793	Other
14				AHDB9790	Other
15	/	Water control	/	Water	Neg control
16	Rotam	Toledo	Tebuconazole (standard)	AHDB9787	Standard/ Positive control
17				AHDB9955	Biocontrol agent

Appendix 2: Orchard trial, treatment application rates (sensitive details removed for coded products)

Product:	Product type	No. of applications	Application rate	Spray volume (L/Ha)	Maximum dose per Ha based on the label
1 (AHDB9891)	Biocontrol Agent	4			
2 (AHDB9791)	Biocontrol Agent	4			
3 (AHDB9936)	Biocontrol Agent	4			
17 (AHDB9955)	Biocontrol Agent	4			
6 (AHDB9794)	Fungicide	2			
7 (AHDB9862)	Fungicide	3			
8 (AHDB9808)	Other	4			
12 (AHDB9792)	Other	4			
15 (Water)	Negative control	4	/	500	/
16 (AHDB9787)	Positive control/ Standard	3	1.2 ml/L	500	600 ml

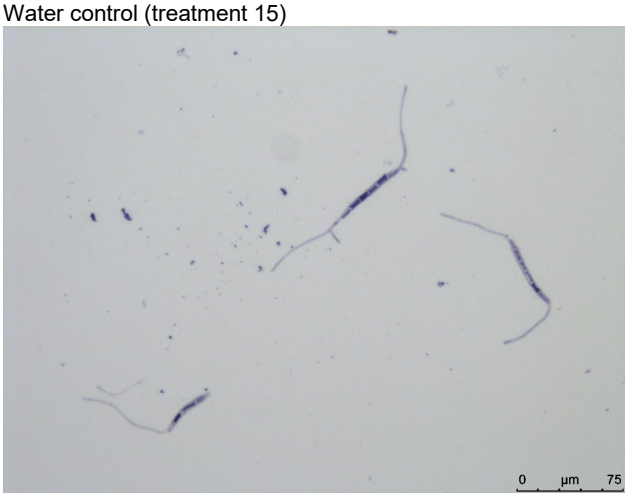
Appendix 3: Trial diary

Date	Event
15/9/20	Product testing against *N. ditissima* Hg199 on PDA plates started.
16/9/20	Product testing against *N. ditissima* Hg199 spore germination on microscope slides.
18/9/20	Product testing plates photographed (day 3)
18/9/20	Trypan blue added to the spore germination slides to stop growth
22/9/20	Mycelial growth plates scanned and measured. Spore germination counts on slides started.
5/10/20	Plots marked and shoots labelled at MP196.
16/10/20	First spray application of all treatments but fungicides
	SP68 plots at MP196: 5 shoots per tree marked for inoculation
26/10/20	SP68 plots at MP196: leaves stripped from all marked shoots to create fresh leaf scars. Wounds created by removing the tip of each shoot (am). All trees in all plots sprayed with all treatments to protect the leaf scars (pm).
27/20/20	SP68 plots at MP196: four leaf scars per shoot inoculated with 4µl of *N. ditissima* macroconidia (9.8 x 10^4 spores/ml). Approximate amounts of isolates used: Hg199 50% R6/20 25% R7/20 25%
28/10/20	*N. ditissima* spore germination rate for the above = 92% after 25 hours in lab conditions.
3/6/21	Canker assessment

Appendix 4: Photos from trial

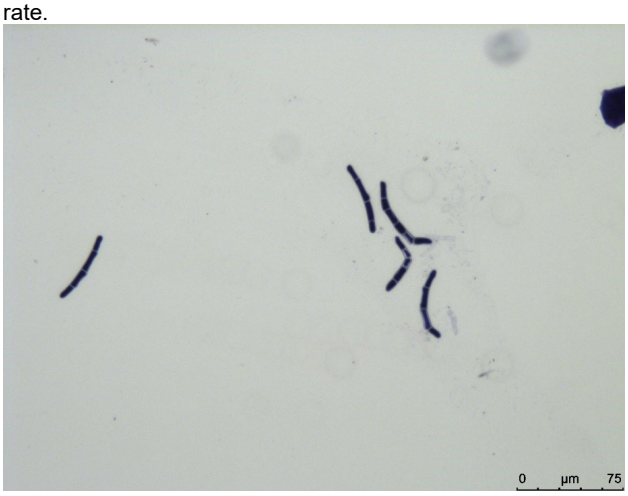
Spore germination assay

Water control (treatment 15)

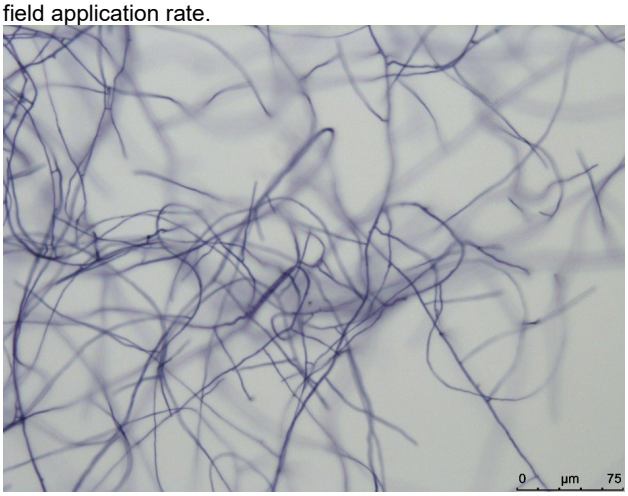


Standard (treatment 16) at field application rate.

Spore germination completely inhibited by treatment 6 (AHDB9794) at field application rate.

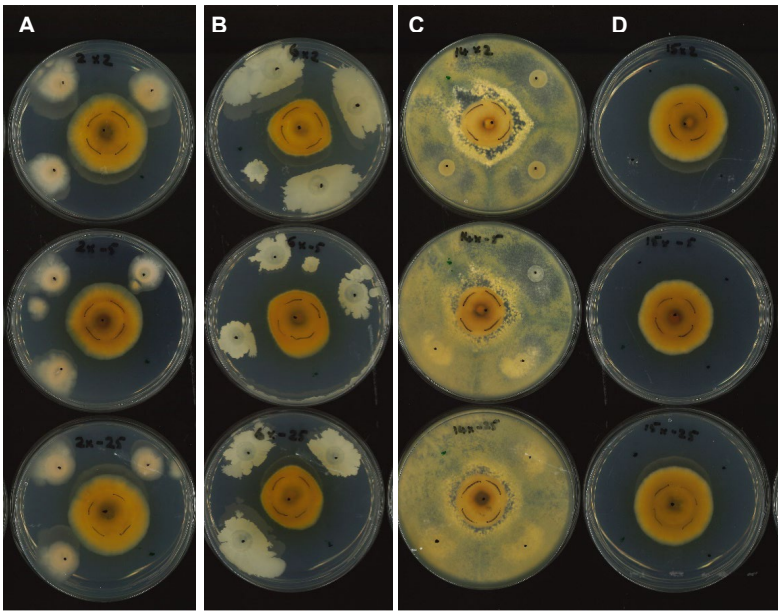


Germination rate and germ tube growth enhanced by treatment 13 (AHDB9793) at field application rate.



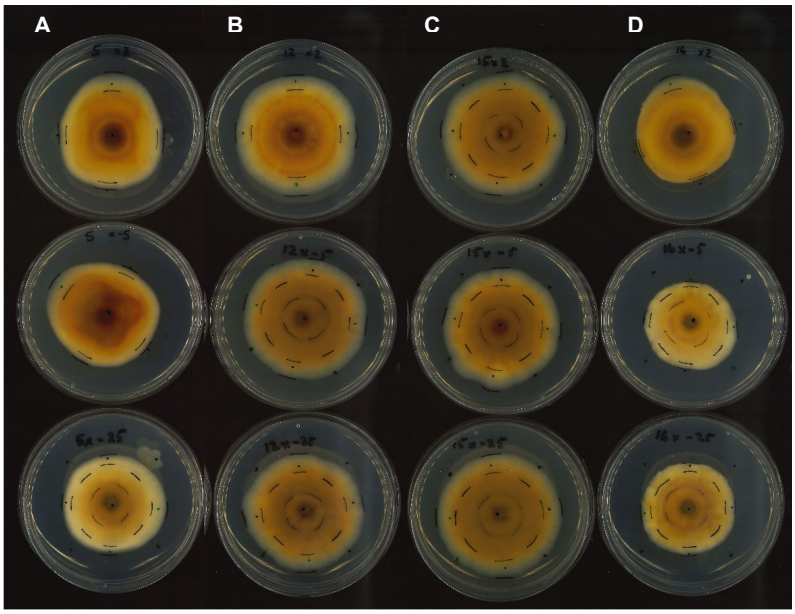
Mycelium growth assay, format 1

A) BCA product with little or no effect on mycelial growth (AHDB9796)
B) BCA product strongly inhibiting mycelial growth by secreting inhibitory substances in the media (AHDB9936)
C) BCA product inhibiting mycelial growth by competition for space and nutrients (AHDB9788)
D) Water control



Mycelium growth assay, format 2

A) Chemical product with strong inhibition on mycelial growth, product 6 (AHDB9794)
B) Product with no effect on mycelial growth
C) Water control
D) Standard (product 16)



Field trial

A) Creating a pruning wound at the tip of the marked shoot
B) Leaves stripped off the marked shoot
C, D) Artificial inoculation with *N. ditissima* spore suspension

Appendix 5: Assessment data

Spore germination data

Mean germination rates, standard deviation (SD) and p-values associated with a two-sided t-test comparing treatments with the water control are listed for each product and each test rate. If a treatment's germination rate on a product at the maximum rate was above ~75% then we did not count the germination rate on the lower application rated. We visually inspected the slides with the lower product rate to confirm that the germination rate was high. These observations are listed as "not counted" (NC).

Prod. no.	Code	Max rate			5x diluted max rate			25x diluted max rate		
		Mean germination (%)	SD	P-val (t-test) vs water	Mean germination (%)	SD	P-val (t-test) vs water	Mean germination (%)	SD	P-val (t-test) vs water
1	AHDB9891	12.22	18.36	0.015	62.17	14.38	NC	95.52	4.37	NC
2	AHDB9791	96.00	2.83	0.343	NC	NC	NC	NC	NC	NC
3	AHDB9936	0.00	0.00	0.010	84.67	8.33	NC	92.67	5.03	NC
4	AHDB9796	96.67	5.77	0.382	100.00	\	NC	93.33	\	NC
5	AHDB9788	96.53	0.68	0.219	NC	NC	NC	NC	NC	NC
6	AHDB9794	0.00	0.00	0.010	4.00	0.00	NC	60.00	2.83	NC
7	AHDB9862	88.67	2.31	0.145	NC	NC	NC	NC	NC	NC
8	AHDB9808	79.96	10.63	0.165	NC	NC	NC	NC	NC	NC
9	AHDB9926	78.40	6.60	0.050	70.00	\	NC	64.00	\	NC
10	AHDB9789	93.73	2.44	0.740	NC	NC	NC	NC	NC	NC
11	AHDB9795	0.00	0.00	0.010	0.00	0.00	NC	0.00	0.00	NC
12	AHDB9792	36.67	32.15	0.093	NC	NC	NC	NC	NC	NC
13	AHDB9793	92.00	8.00	0.873	NC	NC	NC	NC	NC	NC
14	AHDB9790	82.07	19.26	0.435	NC	NC	NC	NC	NC	NC
15	Water	92.86	2.09	1.000	92.86	2.09	1.000	92.86	2.09	1.000
16	AHDB9787	82.88	4.71	0.164	NC	NC	NC	NC	NC	NC

Mycelial growth data

Means, standard error of the means (SEM)) and p-values associated with a two-sided t-test comparing treatments with the water control and standard. Assay formats were: 1) product droplet positioned 2 cm from the edge of growing mycelium, and 2) product droplet positioned directly at the edge of the growing mycelium. The growth after 5 days was measured in both cases.

Assay format	Prod. no.	Code	Max rate				5x diluted max rate				25x diluted max rate			
			Mean growth (mm)	SEM	P-val (t-test) vs Water	P-val (t-test) vs Standard	Average growth (mm)	SEM	P-val (t-test) vs Water	P-val (t-test) vs Standard	Average growth (mm)	SEM	P-val (t-test) vs Water	P-val (t-test) vs Standard
1	1	AHDB9891	3.33	0.47	0.000	0.169	3.00	0.00	0.000	0.000	4.33	0.94	0.008	0.040
1	2	AHDB9791	5.33	0.47	0.000	0.059	5.67	0.47	0.001	0.386	5.00	0.00	0.001	0.074
1	3	AHDB9936	3.33	0.47	0.000	0.169	4.00	0.82	0.005	0.072	3.67	0.47	0.000	0.013
1	4	AHDB9796	9.00	0.00	0.182	0.000	9.00	0.00	0.391	0.000	10.00	0.00	0.182	0.035
1	5	AHDB9788	2.00	0.00	0.000	0.000	2.00	0.00	0.000	0.000	2.00	0.00	0.000	0.013
1	15	Water	9.33	0.47	1.000	0.004	9.33	0.47	1.000	0.010	9.33	0.47	1.000	0.036
1	16	AHDB9787	4.03	0.05	0.000	1.000	6.03	0.05	0.001	1.000	7.00	0.82	0.030	1.000
2	6	AHDB9794	3.00	0.00	0.003	0.000	3.00	0.00	0.003	0.423	4.00	0.00	0.005	0.423
2	7	AHDB9862	3.00	0.00	0.003	0.000	3.33	0.33	0.000	0.230	7.00	0.00	0.038	0.015
2	8	AHDB9808	4.33	0.67	0.011	0.038	3.33	0.33	0.000	0.230	8.00	0.58	0.387	0.010
2	9	AHDB9926	5.33	0.33	0.002	0.006	7.00	0.00	0.038	0.006	7.00	0.58	0.082	0.025
2	10	AHDB9789	5.67	0.33	0.003	0.005	5.67	0.33	0.003	0.003	6.33	0.33	0.008	0.013
2	11	AHDB9795	0.33	0.33	0.000	0.169	7.67	0.67	0.274	0.007	8.00	0.00	0.184	0.008
2	12	AHDB9792	6.67	0.33	0.013	0.003	6.67	0.33	0.013	0.001	8.00	0.00	0.184	0.008
2	13	AHDB9793	9.00	0.00	0.423	0.000	8.00	0.00	0.184	0.004	8.33	0.33	0.519	0.001
2	14	AHDB9790	7.33	0.33	0.047	0.003	8.00	0.00	0.184	0.004	8.33	0.33	0.519	0.001
2	15	Water	8.67	0.33	1.000	0.002	9.33	0.33	0.230	0.000	8.67	0.33	1.000	0.001
2	16	AHDB9787	1.00	0.00	0.002	1.000	2.67	0.33	0.000	1.000	4.33	0.33	0.001	1.000

Orchard trial assessment data

Treatment	Block	Plot no	Wounds with canker	Total wounds	Inoculated leaf scars with canker	Total inoculated leaf scars	Natural inf. leaf scars with canker	Total leaf scars exposed to natural infection
1-AHDB9891	1	4	2	14	4	56	0	197
1-AHDB9891	2	9	0	15	2	60	1	202
1-AHDB9891	3	2	1	11	7	44	7	83
1-AHDB9891	4	3	3	15	26	60	12	212
1-AHDB9891	5	1	0	15	1	60	1	212
1-AHDB9891	6	8	1	12	2	48	0	78
8-AHDB9808	1	10	0	14	7	56	1	178
8-AHDB9808	2	7	0	16	11	64	3	237
8-AHDB9808	3	1	0	14	1	56	1	118
8-AHDB9808	4	9	3	15	7	60	8	223
8-AHDB9808	5	4	1	15	10	60	3	209
8-AHDB9808	6	5	0	7	0	28	0	46
6-AHDB9794	1	3	0	15	0	60	0	187
6-AHDB9794	2	6	1	15	1	60	1	218
6-AHDB9794	3	9	1	15	1	60	0	107
6-AHDB9794	4	10	0	15	0	60	1	213
6-AHDB9794	5	5	1	15	4	60	0	211
6-AHDB9794	6	2	0	15	0	60	0	103
12-AHDB9792	1	7	2	13	4	52	2	167
12-AHDB9792	2	4	0	15	4	60	2	230
12-AHDB9792	3	7	0	13	0	52	0	73
12-AHDB9792	4	8	0	15	2	60	5	217
12-AHDB9792	5	6	2	14	10	56	5	198
12-AHDB9792	6	4	1	13	2	52	1	90
7-AHDB9862	1	5	0	15	1	60	1	213
7-AHDB9862	2	3	0	14	0	56	2	211
7-AHDB9862	3	10	1	12	5	48	1	86
7-AHDB9862	4	5	1	15	9	60	4	226
7-AHDB9862	5	3	0	15	17	60	4	212
7-AHDB9862	6	6	0	14	0	56	0	91
3-AHDB9936	1	1	0	15	0	60	9	210
3-AHDB9936	2	5	0	15	2	60	3	228
3-AHDB9936	3	6	2	13	3	52	3	111
3-AHDB9936	4	4	2	15	10	60	6	230
3-AHDB9936	5	8	3	15	7	60	8	215
3-AHDB9936	6	10	0	14	3	56	6	95
17-AHDB9955	1	8	1	14	6	56	7	196
17-AHDB9955	2	2	1	14	4	56	1	193
17-AHDB9955	3	3	1	11	1	44	0	92
17-AHDB9955	4	7	1	15	9	60	6	224
17-AHDB9955	5	2	0	15	8	60	1	214
17-AHDB9955	6	7	1	13	1	52	4	82
2-AHDB9791	1	6	0	15	17	60	0	207
2-AHDB9791	2	8	0	15	2	60	1	205
2-AHDB9791	3	8	2	12	1	48	0	86
2-AHDB9791	4	1	0	15	11	60	1	201
2-AHDB9791	5	9	2	14	17	56	6	192
2-AHDB9791	6	9	1	14	4	56	0	85
16-AHDB9787	1	9	0	15	0	60	0	199
16-AHDB9787	2	10	1	15	0	60	1	193
16-AHDB9787	3	5	1	15	0	60	0	123
16-AHDB9787	4	6	0	15	0	60	2	217
16-AHDB9787	5	7	2	15	0	60	2	217
16-AHDB9787	6	1	0	14	0	56	0	102
15-Water	1	2	6	15	32	60	7	214
15-Water	2	1	0	15	2	60	3	227
15-Water	3	4	1	8	4	32	0	65
15-Water	4	2	0	15	15	60	2	218
15-Water	5	10	0	13	0	52	2	170
15-Water	6	3	0	12	2	48	1	77

Appendix 6: 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

HSE
Chemicals Regulation Division

Department of Agriculture and Rural Development