

Evaluation of biological and agrichemical products for control of *Neonectria ditissima* conidia production

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Abstract Inoculum control is critical in containing *Neonectria ditissima* but no agrichemical treatments are currently available to protect picking wounds during harvesting. Inhibition of conidia production was tested using a range of chemical-based pruning paints (PP) in a field trial in 2015. Mainly *Bacillus subtilis*-containing, biological products (BP) were tested at the same site in 2016. The BP were also tested in a detached-lesion experiment. Spore-producing field lesions were painted with the products and spore release monitored using glass slides after each rain event. For the detached-lesion study, spore production was monitored using rain traps after weekly artificial rain events. Reduced sporulation was observed in all experiments, but not for all treatments. Some PP created a physical barrier that sealed spores in, with lesions continuing to develop below the paint. No lesion healing was observed from any product in either year, with lesion length increasing for all treatments during the experiments.

Keywords *Cylindrocarpon heteronema*, *Nectria galligena*, conidiation, antisporeulant.

INTRODUCTION

Neonectria ditissima spores, both conidia and ascospores, infect wounds and give rise to European canker lesions in apple trees. In the Tasman region at the top of the South Island of New Zealand, both spore types can be produced all year round and are released during and/or after rain events (Amponsah et al. 2017). Only a few spores (10 or less) are required for wound infection (Walter et al. 2016). The larger the wound and the higher the number of spores reaching the wound, the shorter the latent period from infection to symptom expression (Amponsah et al. 2015; Walter et al. 2016). Depending on wound size and spore load, a latency period as brief as 2–4 months is common (Walter et al.

2016; Amponsah et al. 2015); however, latent periods greater than three years have been reported (McCracken et al. 2003). Inoculum control is a very important aspect in European canker disease control. As the number of spores on a wound increase, so does tissue susceptibility to infection (Dubin & English 1974b; Walter et al. 2016). Inoculum control is primarily achieved by frequent and timely lesion removal, with ongoing monitoring required. In New Zealand, spore release occurs throughout the year as a result of rain, with spore production on lesions peaking in April, coinciding with harvest and hence picking wounds (Amponsah et al. 2015, 2017). No agrichemical treatments are currently

available to protect picking wounds during the harvest period. Sprays of phenylmercury chloride applied during the winter gave a prolonged reduction in sporulation of existing cankers and reduced numbers of leaf-scar infections (Byrde et al. 1952). However, sprayed cankers recovered and sporulation exceeded those of the unsprayed control (Swinburne et al. 1975). Other fungicides tested previously for sporulation suppression have included sodium pentachlorophenate (Wilson 1968), pyridinitril and difolatan (Dubin & English 1974a), benomyl (Bennett 1971; Corbin 1971) and mercuric oxide, othilinone, thiophanate-methyl, and carbendazim gel formulations (McCracken & Cooke 1985). Most of these products are no longer available to be used as sprays in apple production. Carbendazim sprays are not recommended in New Zealand during the autumn period (Walter et al. 2014). In this study, the effects of lesion paints on spore production and spore release were investigated with the goal of reducing the amount of inoculum and hence the risk of infection.

MATERIALS AND METHODS

A series of four experiments were conducted: Field Paint Experiment 1 (FPE1), Field Paint Experiment 2 (FPE2), Detached Lesion Paint Experiment 3 (DLPE3) and Commercial Spray Experiment 4 (CSE4).

Field Paint Experiment 1 (FPE1)

The first field paint experiment was set up in February 2015 in an established, central leader-managed, commercial 'Braeburn' juice orchard in Wills Road, Moutere, near Nelson at the top of the South Island. Nine products, including one biological product, were tested in comparison with an untreated control, with five replicates of each (Table 1). Products were mixed 3–4 h prior to treatment application and held at ambient temperature. Treatments were applied to individual trees on 13 February 2015. Three canker lesions in each tree were selected on 1- to 2-year-old branches with an actively growing tip (i.e. branches were not ring-barked by the lesion). Lesions on two of the branches were painted, ensuring that the lesion and the lesion edges were

covered, while on the third branch one lesion was left exposed as an untreated control. Commercial pruning paints were applied in their supplied bottles with a rubber brush; the other treatments were applied with 4- to 6-cm wide paint brushes. One painted lesion was selected for spore release monitoring. A glass slide coated with petroleum jelly was attached 2–3 cm below the lesion with a bulldog clip held by craft wire. Glass slides were changed after each significant rain event (>2 mm). Spore production of the lesions was determined for two rain events prior to paint application to confirm that the lesions were active and caused by *N. ditissima*. Five slides (air monitoring slides) were placed randomly into the tree rows, not adjacent to lesions, to monitor spores in the air, and thus to determine spore drift from other lesions within the trees. Spores were enumerated by spore type (ascospores and conidia) for three horizontal passes of the slide, one at the outer edge, one in the middle, and one at the clip edge at $\times 200$ magnification. The complete area of the field of view was counted, resulting in a total area of 0.75 cm²/slide. Ring-barking and lesion length were measured at 3- to 4-month intervals. For this experiment, two adjacent rows of 25 trees were used, with trees numbered 1–50 in a serpentine fashion. Treatments were allocated using a Latinised resolvable block design.

Field Paint Experiment 2 (FPE2)

This experiment was carried out to test whether paints containing biological propagules have the potential to reduce the release of *N. ditissima* spores from European canker lesions and/or to assist with lesion healing. Eight biological treatments, a water and an untreated nil control (Table 2) were used, again with five replicates of each. Products were mixed 3–4 h prior to application and held at ambient temperature.

In each tree, on 2- to 4-year-old wood, four lesions were selected (two/tree side) and painted. A large proportion of lesions (69%) had ring-barked the branch by the start of the experiment. Products were applied on 26 April 2016. Treatments were allocated to individual trees in a mature, central leader-managed, 'Royal Gala' juice orchard in Wills Road, Moutere. Treatments

Table 1 Field Paint Experiment 1 (FPE1) in ‘Braeburn’ apple: percentage of European canker lesions where spores were present pre- (two assessments) and post-treatment (ten assessments) and average number of spores counted (post treatment) on glass slides. Dates used for post-treatment exclude the final May 2015 assessment because of missing and damaged slides.

Treatment	Active ingredient	Supplier	Presence pre-treatment (%)	Presence post-treatment (%)	Spores/cm ² post treatment (95% confidence intervals)	Ratio of spores/cm ² post-treatment to control
Acrylic house paint (AHP)	AHP, Dulux Timbacryl	Guthrie Bowron	100	91.4	406.7 (167.5,987.0)**	6.68
Garrison Rapid	2.5 g/litre cyproconazole + 1 g/litre iodocarb	Gro-Chem NZ	90	91.4	79.3 (31.1,202.5)	1.30
Nil control	No paint	-	100	97.1	60.9 (23.7,156.4)	1
Polyvinyl acetate (PVA) + CuO	PVA + 500 g/kg copper oxychloride	Mitre 10 + Yates	100	97.1	37.0 (13.2,103.5)	0.61
AHP + carbendazim	AHP + 500 g/kg carbendazim	Guthrie Bowron + AGPRO NZ Ltd	100	91.4	17.9 (6.3,51.3)*	0.29
AHP + tebuconazole + penetrant	AHP + 10 g/litre tebuconazole + 0.25% polyether modified trisiloxane	Guthrie Bowron + Bayer Corp Science NZ + Etec Crop Solutions	80	68.6	12.5 (4.0,39.0)**	0.21
Fulzyme® Plus	<i>Bacillus subtilis</i> + amino acids	Roots, Shoots & Fruits Ltd	100	85.7	10.2 (3.1,33.3)**	0.17
PVA	Polyvinyl acetate	Mitre 10	100	77.1	7.9 (2.3,26.6)**	0.13
Greenseal Ultra™	10 g/litre tebuconazole + 17.5 g/litre octhlinone	Omnia Nutriology	100	80.0	4.1 (1.0,16.4)***	0.07
Bacseal Super ^A	10 g/litre tebuconazole	Bayer Crop Science NZ	90	48.6	1.9 (0.3,11.6)***	0.03
Random slide ^B	No paint, no lesion	-	-	30.0	3.6	-

^A No longer available.

^B Five random slides were added for the last four assessments to determine potential spore-in-drift; 30% of slides had spores with up to 10 spores/cm² detected; - not tested.

*, **, *** Significantly different from the control treatment at P<0.1, 0.5 and 0.01, respectively.

Table 2 Field Paint Experiment 2 (FPE2), Detached Lesion Experiment (DLE3) products used and product rates thereof. Results for FPE2 ('Royal Gala') and DLE3 ('Scifresh') show number of spores counted on European canker lesions post-treatment (eight and five assessments, respectively) trapped on glass slides and rain traps, respectively.

Product code	Supplier	Product ^A	Active ingredient (a.i.)	a.i. Concentration	FPE2 & DLE3 Product: water ratio	FPE2: Spores/cm ² post-treatment (95% confidence intervals)	DLE3 Spores/lesion post-treatment (95% confidence intervals)
Water control	-	Water	Water only	-	Water only	1085 (539, 2187)	2395 (859, 6674)
Nil control	-	Not treated	No paint	-	Not painted	2266 (1399, 3670)	1454 (542, 3901)
BP 1	Roots, Shoots & Fruits	Superzyme™	<i>Bacillus subtilis</i> <i>Pseudomonas putida</i> <i>Trichoderma koningii</i> <i>T. harzianum</i>	2 × 10 ⁹ cfu/g	1:2 (w/v)	345 (100, 1196)	1271 (468, 3452)
BP 2	Grochem	Clarity	<i>B. subtilis</i> MBI600	5.5 × 10 ⁹ cfu/g	1:2 (w/v)	351 (103, 1204)	405 (148, 1112)
BP 3	Bayer CropScience	Serenade® Optimum	<i>B. subtilis</i> QST713	26.2%	1:2 (w/v)	552 (207, 1473)	229 (84, 624)
BP 4	Etec Crop Solutions Ltd	Bacstar™	<i>B. subtilis var. amyloliquefaciens</i> D747	5 × 10 ¹⁰ cfu/g	1:2 (w/v)	774 (338, 1775)	831 (307, 2254)
BP 5	Etec Crop Solutions Ltd	Bacstar™	<i>B. subtilis var. amyloliquefaciens</i> D747	5 × 10 ¹⁰ cfu/g	1:10 (w/v)	535 (197, 1452)	903 (333, 2448)
BP 6	Roots, Shoots & Fruits	Fulzyme® Plus	<i>Bacillus subtilis</i> + amino acids	2 × 10 ¹⁰ cfu/mL	1:1 (v/v)	835 (376, 1856)	333 (122, 908)
BP 7	BioStart Ltd	µInoculant PP9 ^B	<i>P. putida</i> Ps1	> 10 ⁹ cfu/mL	1:1 (v/v)	1822 (1061, 3129)	366 (135, 992)
BP 8	Biostart Ltd	TripleX®	<i>B. amyloliquefaciens</i> Bs1b	> 10 ⁶ cfu/mL	1:1 (v/v)	1519 (840, 2747)	2244 (832, 6057)
Random slide	-	Random slide ^C	No paint, no lesion	-	-	0-13	-

^A Product code and simple product names used in the text to differentiate between the Biological products (BP) more easily, instead of listing the often long and complex active ingredients

^B Foliacin added as a co-substrate at a rate of 10 mL/litre. Supplied by Biostart Ltd.

^C Five random slides were added to determine potential spore-in-drift in FPE2. The minimum and maximum number of spores counted are given.

were applied after spore-release monitoring for two rain events as described in FPE1. Spore release was monitored as described earlier; however, plain glass slides (i.e. not petroleum jelly-coated) were used. In this experiment, the 50 trees were selected from two adjacent rows, with the trial laid out as a Latinised resolvable block design. Prior to paint application, 10 trees with high spore counts (>200 spores/slide count) were identified. To ensure reasonably even distribution of these trees across treatments, 999 randomisations of the design were generated. Of these, those with each treatment having exactly one replicate on a high-spore-count tree were identified (five of the 999), and one of these was randomly selected to be used for the trial.

Detached Lesion Paint Experiment 3 (DLPE3)

This experiment was conducted in parallel to FPE2 using 50 detached 'Scifresh'/Jazz® twig sections with lesions (1- to 2-year-old wood) collected from a commercial orchard. The same treatments were used (Table 2) and products were mixed 3–4 hours prior to use. The experiment was set up to determine total spore release under more temperate/warmer conditions. Spores were washed from the lesion with the aid of four irrigation sprinklers, to simulate rain. The sprinklers were mounted 80 cm above the lesions. These 50 stem sections were suspended on five metal rods (2-m long). Sprinklers were set up in the corners of the frame (2 m × 2 m), with the rods located in the centre of the quadrant. Treatments were arranged in a randomised block design with a full set of treatments per rod. Before each weekly irrigation event, lesions were re-randomised within each rod to compensate for variation among irrigation intensities at the various positions. Spores were collected in Falcon tubes (50 mL) placed directly underneath the lesion during the artificial rain event of approximately 30 min. One to two rods were irrigated together with rods 10–15 cm apart and lesions also spaced 10–15 cm apart on each rod. After the irrigation, lesions were allowed to drip dry and were then incubated on the rods in the laboratory at 15–18°C. Water volume collected during the artificial rain event was determined

by weight, and spores were enumerated using a haemocytometer. The first simulated rain event was on 22 April 2016; this was then repeated for five weeks in 6- to 8-day intervals.

Commercial Spray Experiment 4 (CSE4)

This experiment was conducted in 'Royal Gala' and 'Granny Smith' orchard blocks located in the Waikato region of the North Island. Spore release was measured using plain glass slides sprayed with and without *Bacillus subtilis* (Fulzyme® Plus) at 1 litre product/ha applied as a fine mist during light rain or before rain at 2000 L/ha water rate. Product applications were made on: 20, 31 October; 8, 19 November; 5, 9, 23 December 2016; and 13 January 2017. In each cultivar and treatment (with or without *B. subtilis*) block, five European canker lesions were selected from two adjacent rows, with at least three buffer rows between treatments. Lesion length was measured. Slides were placed and exchanged on: 31 October; 7, 14, 21, 28 November; 5, 12, 26 December 2016; and 2, 9, 16, 23 and 31 January 2017. Spore density was counted as described earlier.

Statistical analyses

Initial analyses in FPE1 and FPE2 were conducted to determine spatial effects by replicate, tree row or row position. In most cases, the effect of replicate was not significant and neither was that of tree row. However, the effect of individual trees was significant (since measurements taken from the same tree tend to be more highly related than those from separate trees), therefore 'Tree' was included as a random effect in further analyses.

There was some inconsistency between people in assessing lesion length and degree of ring-barking. Thus, data for each length assessment were examined separately with analysis of variance (ANOVA). The percentage of lesions that were ring-barked was initially analysed using a hierarchical generalised linear model (HGLM, Lee et al. 2006), with a binomial distribution for the fixed effects (Treatment) and a beta distribution for the random effects (Tree), both with logistic links. However, since the 'Tree' random effect was found to be negligible, the data as presented were analysed with a binomial

generalised linear model, with a logit link (GLM, McCullagh & Nelder 1989). The dispersion was estimated.

For spore counts, the sum of conidia and ascospore counts is presented: conidia counts dominated these. The data were summarised over the pre-treatment (sum of counts over assessments 1 and 2), and also over the post-treatment (sum of counts over assessments 3 to 10 (FPE1) and 3 to 8 (FPE2)). These two sets were analysed separately using a Poisson GLM with a logarithmic link. The dispersion was estimated.

In DLPE3, two analyses were carried out, one using the data for the pre-treatment assessments, and the other for all post-treatment assessments. To give an analysis of totals over the post-treatment assessments, HGLM, with a Poisson distribution for the fixed effects (Treatment) and a gamma distribution for the random effects (Lesion, assessment within lesion), both with logarithmic links, were used. The model included an offset (McCullagh & Nelder 1989) of $-\log(10^3 \times \text{Wash Volume})$ to convert results into counts/mL. Predicted means were obtained using an offset of 0, which were then multiplied by the number of assessments to give the totals over assessments. The analyses included an overall test for treatment differences, which was an F-test (ANOVA, GLM with dispersion estimated), or a χ^2 test (GLM with dispersion not estimated; HGLM analyses). All analyses were carried out with GenStat (GenStat Committee 2015).

The CSE4 data have been averaged for the five lesion counts per cultivar and treatment. Data have been summarised descriptively for all 13 assessments.

RESULTS AND DISCUSSION

Field Paint Experiment 1 (FPE1)

Spore in-drift on random slides was low (Table 1). Forty-seven of the 50 cankers monitored using petroleum jelly-coated glass slides released spores on both assessment dates before paints were applied. Of the other three, two released spores on one of the assessments only, and spores were not captured for the third. The spore release pattern (Table 1) was repeated in each of the assessments (data not shown). At all assessments

except the last (assessment 10, 7 May 2015; $P=0.192$), there were significant differences in spore release between treatments ($P<0.025$ or smaller). Spore release was highest between 23 March and 27 April 2015.

There were substantial differences in spore numbers between the treatments ($P<0.001$). The acrylic house paint (AHP) alone significantly increased spore release compared with that in the untreated control. However, the addition of carbendazim or tebuconazole to AHP showed a reduction in overall spore release. Polyvinyl acetate (PVA) alone also functioned as a spore release barrier, but to a lesser extent if amended with copper oxychloride. The two tebuconazole-containing commercial pruning paints provided a seal over the lesions with no or few spores found on glass slides underneath those cankers.

From the 200 branches used in FPE1, only four showed any signs of healing. In May, the average lesion length of painted and non-painted lesions was 37.9 mm, with no significant differences in lesion length among the paint treatments ($P=0.132$). In August and November, the average lesion length was 52.1 mm, with differences among treatments not varying significantly ($P=0.447$ and $P=0.988$, respectively). Differences in lesion length between painted and non-painted lesions were not significant in any of the assessments ($P>0.3$), but ongoing lesion length expansion occurred for both painted and non-painted lesions ($P<0.001$). Lesion length expansion was correlated to ring-barking (i.e. lesion width expansion). None of the 200 lesions selected in February was ring-barked. However, in May 40% of shoots were ring-barked, increasing to 67% and 81% in August and November, respectively. However, differences between painted and non-painted shoots, and among treatments were not significant ($P>0.1$).

Except for the control and *B. subtilis* treatments, all others contained paint polymers. The *B. subtilis* treatment was an aqueous solution. Reduction in spore release by the polymer-based paints was largely due to the physical barrier of the paint. When stripping back the polymer seal at the end of the experiment, abundant sporodochia were observed beneath the paint.

Because of the reduction in spore release by the *B. subtilis* treatment (83%) compared with that in the control, further research on biological products (BP) as anti-sporulants was conducted.

Field Paint Experiment 2 (FPE2)

Air monitoring slides yielded very few spores (Table 2). Ascospores comprised fewer than 6% of all spores counted (3362 of 61,197 total), with 200 of the 500 data values equal to 0. Thus, patterns observed in total counts were predominantly of conidia. Therefore, data are shown for total spores only.

Total spore numbers did not vary substantially between the treatments ($P=0.985$) before treatment. However, after treatment, mean numbers varied substantially ($P=0.006$; Table 2). The number of spores captured was highest for the Nil control at 2266 spores/cm², and lowest for BP1 (Superzyme™) and BP2 (Clarity), with approximately a sixth as many spores counted, at 346 spores/cm² and 351 spores/cm², respectively. The next highest spore counts, after the Nil control treatment, were recorded from lesions treated with BP7 (PP9, 1822 spores/cm²) and BP8 (Triplex, 1519 spores/cm²), which in both instances higher than those for the Water control application (1058 spores/cm²). Differences in spore release could be observed at most assessment times after treatment, with similar patterns amongst the treatments (data not shown). Spore release was high and remained similar for both controls throughout the study.

The average lesion length increased by approximately 15.8 mm during the course of the experiment (after treatment application), with no significant differences between treatments ($P=0.936$). The proportion of branches that were ring-barked by the lesions at the onset of the experiment was 62%, and increased only slightly to 69% from April to September 2016, with no significant differences between treatments ($P=0.277$).

Detached Lesion Experiment 3 (DLE3)

The average quantity of water captured in the rain traps from simulated rain ranged from 7.2 to 11.2 mm for all lesions and treatments. However,

as little as 2.4 mm and as much 35.4 mm of water was trapped for individual lesion washes (depending on lesion and twig size as well as position during the irrigation event). Hence spore data are presented as total spores/lesion.

Before treatment, the number of spores per lesion varied from 497 spores for the Nil control, to 1452 spores for BP6 (Fulzyme®), a nearly three-fold range, although this difference in treatments was not statistically significant ($P=0.664$). After treatment, the number of spores averaged over all assessments varied substantially between treatments ($P=0.008$, Table 2), ranging from 229 spores/lesion for BP3 (Serenade®) to 2395 spores/lesion for the Water control (10.4-fold range). Spore counts per lesion for BP6 (Fulzyme®) and BP3 (Serenade®), BP2 (Clarity) and BP7 (PP9) were significantly lower than those for the Nil control and the Water control (means below 20% of the mean for Water). At the last wash (2 July 2016), very few conidia were recovered in all treatments because perithecia were observed to begin to form.

The effects of lesion paints in the field (FPE1, FPE2) and laboratory (DLE3) experiments are compared in Table 3. Only *B. subtilis* BP6 (Fulzyme®) applied at a 1:1 (v/v) ratio was used in all three experiments. The reduction in sporulation (compared with that in the Nil control) was 83.2%, 63.1%, 77.1% in FPE1, FPE2 and DLE3, respectively. In FPE2, the reduction in sporulation for the other *Bacillus*-containing products was similar, ranging from 84.7% to 65.8%.

Reduction in sporulation by biological products, particularly those containing *B. subtilis*, compared with the untreated Nil control, was observed in all lesion paint experiments (FPE1, FPE2, DLE3). In FPE1, painting lesions with *B. subtilis* reduced spore release by 83% compared with that in the Nil control treatment. The microbial-containing products applied in FPE2 and DLE3 also reduced spore release. However, there were clear differences in ranking spore release after application of products between the field and the laboratory-based assay. For example, BP7 (PP9) did not reduce spore release in the field, but did in the laboratory. In contrast, application of the fungal-bacterial mix in BP1 (Superzyme™)

Table 3 Sporulation potential (%) based on the untreated control from European canker lesions painted with biological products (BP) in Field Paint Experiment 1 (FPE1), Field Paint Experiment 2 (FPE2), and Detached Lesion Experiment (DLE3).

Treatment code	Product ^A	FPE1 % (spores/cm ²)	FPE2 % (spores/cm ²)	DLE3 % (spores/lesion)
Nil control	Not treated	100.0 (60.9)	100.0 (2266.1)	100.0 (1454.4)
BP 1	Superzyme™	-	15.3 (345.6) **	87.4 (1270.5)
BP 2	Clarity	-	15.5 (351.2) **	27.8 (404.9) *
BP 3	Serenade®	-	24.4 (552.0) **	15.7 (228.9) **
BP 4	Bacstar™ 1:2	-	34.2 (774.6) *	57.2 (831.4)
BP 5	Bacstar™ 1:10	-	23.6 (535.4) **	62.1 (902.5)
BP 6	Fulzyme®	16.8 (10.2) **	36.9 (835.2) *	22.9 (332.7) *
BP 7	PP9	-	80.4 (1822.0)	25.1 (365.6) *
BP 8	TripleX®	-	67.0 (1519.2)	154.3 (2244.4)
Water control	Water	-	47.9 (1085.2) *	164.7 (2394.9)

^A Described in Table 2

- Not tested

*, ** Significantly different from the Nil control at P<0.05 and P<0.01 respectively

resulted in a reduction of spore release in the field, but not in the laboratory. Equally, the large spore release reduction by BP6 (Fulzyme®) in FPE1 was not repeated in FPE2, but was in DLE3.

Temperature drivers may explain the differences in sporulation control amongst the experiments. In FPE1, treatments were applied in February (late summer), in FPE2 in April (mid-autumn), and in the laboratory the average temperature was approximately 18°C (equivalent to early harvest). For example, the bacterium *P. putida* prefers warmer conditions, with an optimal growth rate at 30°C, with temperature influencing its transcriptome and proteome profile (Fonseca et al. 2011). Similarly, *B. subtilis* is able to sustain growth from 11 to 52°C, with an optimum growth rate between 30 and 37°C, and its transcriptome and proteome profile is also temperature dependent (Budde et al. 2006). Hence, the effect of temperature needs to be considered, particularly for organisms that are well adapted to warmer (>25°C) temperatures. Nevertheless, the *B. subtilis* products reduced spore release from European canker lesions overall. In these experiments, irrespective of strain type (Table

2), formulation and/or rate of product applied (1:1, 1:2 or 1:10), the biological products were all more or less equally effective in reducing spore release. These results are promising; however, the effects of temperature and lower product concentrations need further research. Similarly, strain variations and formulations should be further explored.

McCracken & Cooke (1985) reported that only mercuric oxide reduced lesion expansion and a carbendazim-triadimefon-xanthan gel stimulated callus formation. Neither in FPE1 nor in FPE2 did the painting treatments facilitate lesion healing as measured by lesion expansion and ring-barking. Lesion extension was slower during the non-dormant tree phase than during the dormant tree phase, but this could also be driven by climatic parameters. Interactions among plant defence, climate and plant physiology, however, are likely.

For fungicide-containing pruning paints, painting lesions on branches with many fruit was difficult without contaminating fruit. Paints dripping onto fruit or accidentally applied to fruit may cause residues. If paints containing chemical fungicides are to be used in season for

sporulation control, registration will be required under the Agricultural Compounds & Veterinary Medicines (ACVM) regulations.

Commercial Spray Experiment 4 (CSE4)

From the 13 slide collection times, all but four yielded spores on glass slides (Figure 1). For these four collection dates, there was no rain during the exposure period. The number of spores trapped on glass slides underneath European canker lesions overall was five-fold higher for ‘Royal Gala’ (285 spores/cm²) than for ‘Granny Smith’ (59 spores/cm²). Slightly fewer spores were trapped from lesions sprayed with *B. subtilis* (134 spores/cm²) than in the control treatments (211 spores/cm²), approximately a 36% reduction in spore numbers. In earlier experiments (Walter et al. 2016), only 3–10 spores were required for wound infection and symptom development.

As only a few spores are required for infection (Walter et al. 2016), we hypothesise that similar rates of infections to those in untreated controls will occur. To that effect, field studies are in progress. Indeed, *B. subtilis* and other biological products have not provided wound protection (on susceptible cultivars (Walter et al. 2017; Swinburne & Brown 1976).

CONCLUSION

Painting actively growing European canker lesions can provide control of spore release, depending on the product used. This, however, is only a short-

term solution as lesions continue to expand and eventually breach or outgrow the painted area. Wound paint is, however, a tool to be considered for inoculum control, if and where pruning is not possible. The risk of fruit contamination with chemical paints also needs to be considered and fungicide-based paints should not be used during the fruiting season because of risk of residues. In New Zealand, for example, fungicide containing pruning paints are not registered for in-season use. In these three experiments, products containing *B. subtilis* and/or *P. putida* showed great promise when applied at high concentrations as anti-sporulants, particularly during warmer conditions. In our studies, the products were painted onto lesions at high concentrations of active ingredient. Spray application at lower rates or at rates currently used in pipfruit (CSE4) provided little benefit for sporulation control. This may have been due to poor deposition onto European canker lesions as well as propagule concentration. The effect of temperature and propagule concentration for sporulation control needs to be further investigated to determine performance limitations of potential microbial anti-sporulants *in situ*.

The use of *B. subtilis* and other microbial products as anti-sporulants and potential wound protectors at varying product concentrations should be further explored for use during the harvest season.

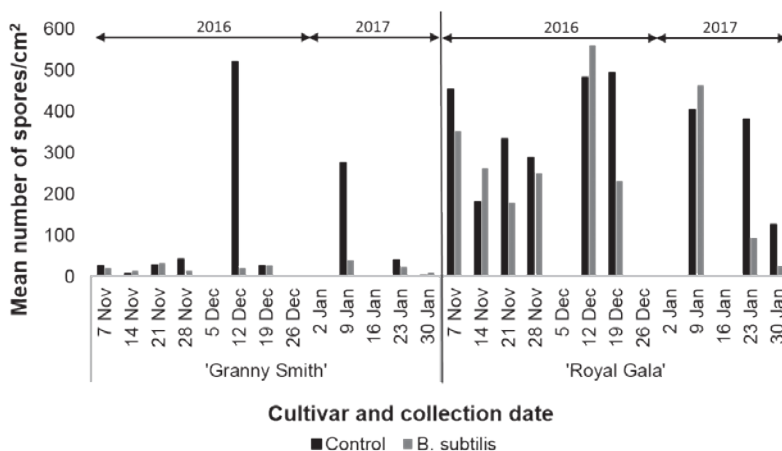


Figure 1 Mean *Neonectria ditissima* conidia counts on glass slides (mean number of spores/cm²) underneath European canker lesions in commercial ‘Granny Smith’ and ‘Royal Gala’ orchard blocks in the Waikato region. Trees were sprayed without (Control) and with (*B. subtilis*) *Bacillus subtilis* (Fulzyme® Plus) at 1 litre/ha product rate using a 2000 litre/ha water rate.

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