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Efficacy and optimal timing of low-volume aerial applications of copper fungicides for the control of red needle cast of pine

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Abstract

Background: Red needle cast (RNC) is a foliar disease of radiata pine (*Pinus radiata* D.Don), caused by *Phytophthora pluvialis* Reeser, Sutton & E.Hansen and occasionally *Phytophthora kernoviae* Brasier, Beales & S.A.Kirk. The disease has impacted plantations in New Zealand since at least 2008. To develop management recommendations for red needle cast, research has focused on identifying chemical control options and understanding pathogen epidemiology to guide optimal timing of spray application. The objectives of this study were to: (1) assess the efficacy of aerial copper fungicide application for the control of red needle cast in mature radiata pine plantations; and (2) investigate optimal spray timing.

Methods: To address these objectives, three operational-scale field trials were undertaken in successive years between 2017 and 2019 at a forest in the Central North Island of New Zealand. RNC severity was assessed in canopies of forest blocks exposed to cuprous oxide applied at 0.855 kg ha^{-1} active ingredient in low-volume aerial spray at different times of the year (November, February and April (or May)). Needle cast from plantation trees and infection levels on trap plants were also assessed in some years.

Results: Application of cuprous oxide significantly reduced RNC severity in all three trials. As well as reducing disease severity, application of cuprous oxide also tended to reduce needle cast from plantation trees and infection on trap plants in years when these were also assessed. No consistent effect of spray timing was observed. Generally, all three spray timings reduced disease severity compared to the unsprayed control, but differences were not always significant, and few differences were detected between different spray timings.

Conclusions: The results reported here are the first to show that low-volume aerial applications of cuprous oxide applied at 0.855 kg ha^{-1} active ingredient can reduce the severity of RNC in commercial radiata pine plantations. No consistent effect of spray timing was detected. These findings support the development of management recommendations for RNC.

Keywords: Disease control, disease management, foliar *Phytophthora*, oomycete, pathogen, pine needle disease

Introduction

Red needle cast (RNC) is a foliar disease of radiata pine (*Pinus radiata* D.Don), caused by *Phytophthora pluvialis* Reeser, Sutton & E.Hansen and occasionally *Phytophthora kernoviae* Brasier, Beales & S.A. Kirk, that has impacted plantations in New Zealand since at least 2008 (Dick et al. 2014). Olive coloured lesions on needles, that may contain black, resinous bands, are characteristic early symptoms of the disease (Dick et al. 2014). Lesions quickly turn khaki in colour and affected needles become yellow or red, before being readily cast (Dick et al. 2014). The timing of disease expression varies between sites and years, but generally the first symptoms will be seen in autumn or winter on the lower branches of infected trees. Under conducive conditions, the disease will rapidly spread upwards within affected trees and to neighbouring trees, and affected needle fascicles will be cast by early-mid spring (Dick et al. 2014; Fraser et al.

2020). Under favourable conditions, affected trees that were completely green at the start of autumn can be nearly completely defoliated by spring, but new spring growth is seldom affected (Dick et al. 2014).

Both *Phytophthora* species associated with RNC are also found associated with other host species and in other regions of the World. *Phytophthora pluvialis* is thought to be native to the Pacific Northwest region of North America (Brar et al. 2018; Tabima et al. 2021), where it was first described from cultures isolated from stream, soil and canopy drip baitings in mixed tanoak-Douglas fir (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H.Oh-*Pseudotsuga menziesii* (Mirb.) Franco) forests and from twig and stem cankers on tanoak (Reeser et al. 2013). *Phytophthora pluvialis* also causes RNC on Douglas fir in New Zealand and in the Pacific Northwest region of North America (Hansen et al. 2015). More recently, *P. pluvialis* has been reported from the United Kingdom (UK) on western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), causing crown dieback, needle drop, and branch and stem cankers on mature trees, as well as mortality of young trees (Pérez-Sierra et al. 2022).

Phytophthora kernoviae was originally described from cultures isolated from European beech (*Fagus sylvatica* L.) and *Rhododendron ponticum* L. in the UK (Brasier et al. 2005), but is thought to be native to New Zealand (Gardner et al. 2015; Studholme et al. 2018) where it is known to have been present since at least 1953 (Ramsfield et al. 2007). In New Zealand, the pathogen was first isolated from soils beneath mature radiata pine that had experienced "marked but shortlived outbreaks of foliar reddening" in Kinleith Forest, Central North Island (McAlonan 1970). Recent analyses of herbarium and archive material demonstrates that *P. kernoviae* has been infecting radiata pine foliage since at least the 1980s and that it may have been the cause of a previously unexplained disorder called physiological needle blight (McDougal & Ganley 2021). *Phytophthora kernoviae* has also been reported from the Republic of Ireland (Brennan et al. 2010) and Chile (Sanfuentes et al. 2014). It has a wider known host range than *P. pluvialis* and has been isolated from, or found in association with, a wide range of angiosperm, gymnosperm and fern families that are listed in Fraser et al. (2020).

Before *P. pluvialis* arrived in New Zealand and RNC emerged as a problem, the main needle disease affecting radiata pine plantations was Dothistroma needle blight (DNB), caused by the ascomycete fungus *Dothistroma septosporum* (Dorogin) Morelet (Drenkhan et al. 2016). This disease has been managed in New Zealand with low-volume aerial applications of copper-based fungicides for over 50 years (Bulman et al. 2016). For control of DNB, aerial disease surveys are undertaken annually in mid-winter (June and July) and if disease severity passes a certain threshold (20% for most forestry companies), copper is applied in late spring or early summer (October or November) to protect newly emerging needles from infection. If severity is deemed high enough (>40% for most companies), a second spray in late summer (February) may also be applied (Bulman et al. 2016). Advances in spray technology over the years have enabled reductions in application rates to 0.855 kg ha^{-1} active ingredient in 2 L of oil diluted to 5 L ha^{-1} with water (Bulman et al., 2004). Copper deposits on treated needles slowly dissolve in water and spread over needle surfaces and act against *D. septosporum* in two ways: (1) by killing conidia (spores) released during wet periods thus protecting treated needles from infection (Franich 1988); and (2) by inhibiting conidiomata (fruiting bodies) from producing and releasing new conidia thus reducing inoculum loading (Bulman et al. 2013).

To develop management recommendations for RNC in existing radiata pine plantations, research has focused on identifying chemical control options and understanding pathogen epidemiology to guide optimal timing of spray application. Early experiments with *P. pluvialis* and *P. kernoviae* demonstrated that copper fungicides inhibited mycelial growth and sporulation of both species in culture (Rolando et al. 2017). Detached needle assays further demonstrated that treatment of young radiata pine plants with copper oxychloride protected needles from infection by both pathogens for at least three months (Rolando et al. 2017). More recently, controlled artificial inoculation experiments with intact 18-month-old rooted cuttings of radiata pine demonstrated that cuprous oxide applied at the industry standard rate to control DNB also protected needles from infection by *P. pluvialis* for at least 3 months (Rolando et al. 2019). The same was found in artificial inoculations of detached needles collected from operationally sprayed field trials in commercial plantations (Rolando et al. 2019). However, until now, the impact of operational-scale copper sprays on the development of RNC within radiata pine plantations has not been investigated.

Research on the epidemiology and biology of *P. pluvialis* and *P. kernoviae* on radiata pine has been undertaken since the discovery of RNC in 2008. Both species are polycyclic pathogens, capable of several infection cycles per season (Fraser et al. 2020; Gomez-Gallego et al. 2019). Under optimum conditions, *P. pluvialis* can sporulate within three days of infection, demonstrating how epidemics are able to develop so rapidly within affected stands (Gomez-Gallego et al. 2019). Although there is variation between years and sites, detection of inoculum of both species across seasons follows a similar pattern to symptom development in New Zealand radiata pine plantations. Inoculum is predominantly detected in the coolest and wettest months of the year, with a peak between June and August, and little detection in the warmer and drier summer months (Fraser et al. 2020). A similar pattern has also been seen for infection (Hood et al. 2022). It has been suggested therefore that copper applications in autumn (March-May), which protect needles for the first three months of the RNC season, may be most effective (Fraser et al. 2020; Hood et al. 2022; Rolando et al. 2019) with potential benefits from a second application three months later.

The objectives of this study were to: (1) assess the efficacy of aerial application of cuprous oxide for the control of RNC in mature radiata pine plantations; and

(2) investigate optimal spray timing to maximise control. To address these objectives, three operational-scale field trials were undertaken in successive years between 2017 and 2019 at a forest in the Central North Island of New Zealand. RNC severity was assessed in canopies of forest blocks that were exposed to various cuprous oxide spray timing treatments or left unsprayed. Needle cast from plantation trees and infection levels on trap plants were also assessed in some years.

Methods

Three operational-scale trials were undertaken between 2017-2019 at Kinleith Forest in the Central North Island, New Zealand (Fig. 1). The trials were undertaken in mature stands, established between 1993 and 1999, in an area of the forest where RNC outbreaks had occurred in previous years. Treated blocks were sprayed by helicopter with cuprous oxide applied in very fine droplets (VMD of 80 µm using Micronair nozzles). The

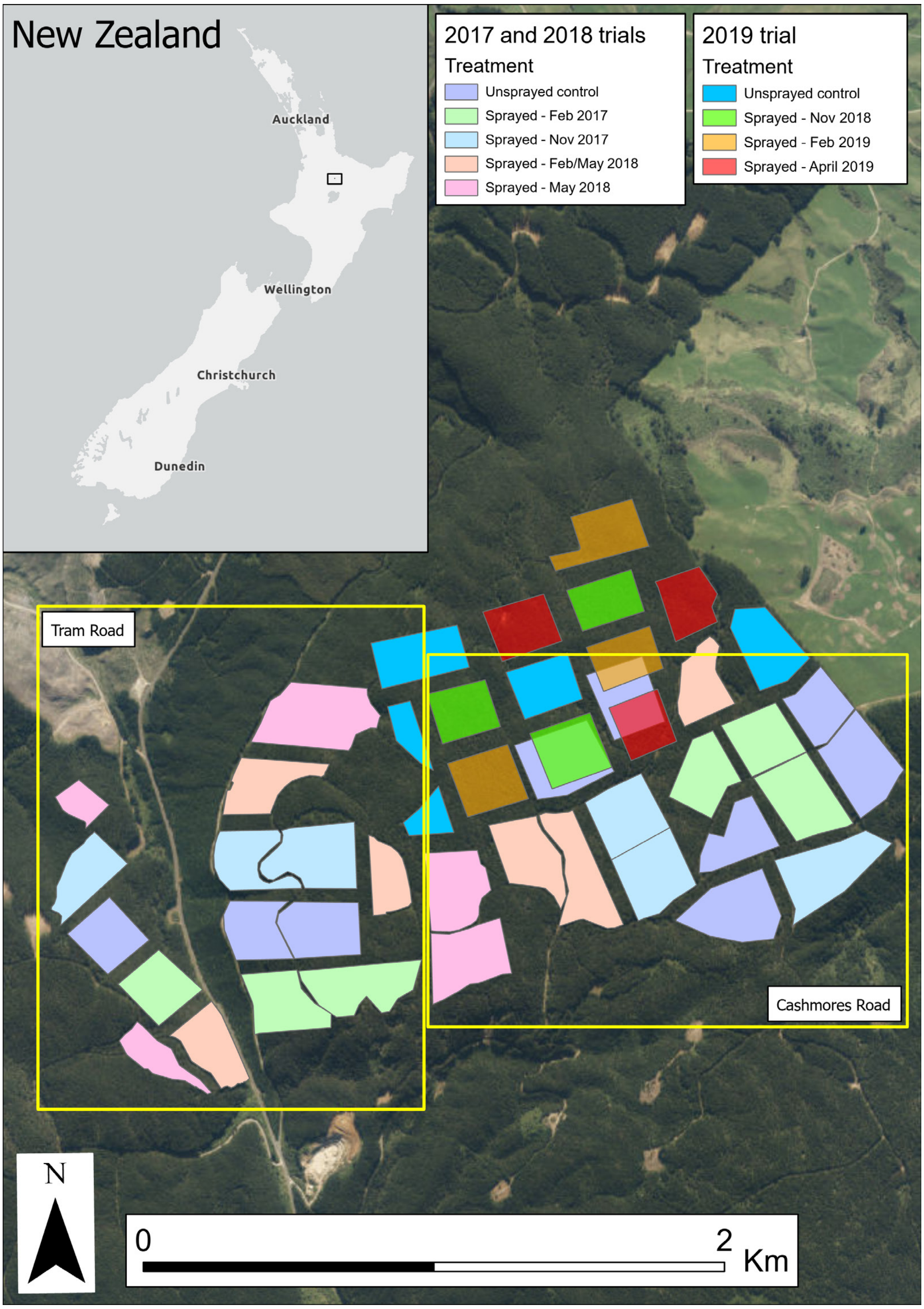

FIGURE 1: Location and layout of trials to test the efficacy of low-volume aerial applications of cuprous oxide on red needle cast.

cuprous oxide was applied at a rate of 0.855 kg ha^{-1} active ingredient in a total volume of 5 L ha^{-1} (comprising 2 L crop oil, with water added to make total volume of 5 L), as per standard for the control of DNB (Bulman et al. 2016). The 2017 trial had one treatment timing, while the 2018 and 2019 trials included a spray timing component in the experimental design (Table 1).

2017 Trial

Trial layout and treatment

The 2017 trial was undertaken at two sites, Tram Road and Cashmore's Road. Three blocks (c. 4 ha) were sprayed at each site on 11 Feb 2017, and three blocks were left as unsprayed controls. At the Tram Road site, blocks were situated in stands aged 18, 23 and 24 years, with one sprayed and one control block per year of planting. At the Cashmore's Road site, all blocks were situated in stands aged 24 years.

Disease assessments

The severity of symptoms in tree canopies was assessed visually from the ground on 14 September 2017. A transect of twenty trees was assessed along road edges of each block. The percentage of the canopy of each tree with symptoms of RNC was estimated to the nearest 5% level.

Quantification of needle cast

Three traps to collect cast needles were placed within each block on 31 July 2017 at the Cashmore's Road site and 14 August 2017 at the Tram Road site (36 traps in total). Vegetation was cleared around each trap to prevent interception of cast needles by overhanging or neighbouring branches. Traps were placed along transects in the centre of each block, with at least 20 m between traps. Traps consisted of wooden or plastic frames lined with fine mesh (c. 2 mm^2 pore size), with a surface area of c. 0.36 m^2 at Cashmore's Road and c. 0.23 m^2 at Tram Road. Cast needles were collected every 2-3 weeks until 26 October 2017. Needles were dried and weighed, and dry weights adjusted to g m^{-2} to account for the variation in trap size. Daily adjusted dry weights (g m^{-2} day^{-1}) were calculated by dividing the adjusted dry weights by the number of days that the traps were in the field. Before the needles were dried on 14 September, up to 10 still partially-green needles per trap were sampled and prepared for species-specific qPCR analysis following the method outlined by O'Neill et al. (2018) (see below).

TABLE 1: Details of cuprous oxide treatments tested, and red needle cast disease assessments applied in each trial.

	Trial Year		
	2017	**2018**	**2019**
Number of Sites	2	2	1
Copper treatments			
Unsprayed control	✓	✓	✓
November spray		✓	✓
February spray	✓		✓
February + May spray		✓	
May spray		✓	✓
Disease assessments			
Ground-based assessments	✓	✓	✓
Aerial assessments		✓	✓
Quantification of needle cast	✓		✓
Pathogen inoculum quantification and identification with trap plants	✓	✓	

Pathogen identification and inoculum load

Six trap plants were placed within each block on 31 July 2017 (or on 14 August for two blocks at Tram Road) to identify the species of *Phytophthora* present and to assess inoculum load in each block. Trap plants were grafted cuttings of RNC-susceptible radiata pine genotypes c. 50-100 cm in height in plastic pots. They were placed at 10 m spacing along a transect running through the centre of each block. Needle samples from all plants with symptoms of *Phytophthora* infection were collected on 31 August (or on 14 September for the two blocks with delayed deployment at Tram Road), 5 October and 26 October and isolations attempted and/or qPCR undertaken. Disease severity was scored on the trap plants on 5 October by estimating the percentage of needles with symptoms to the nearest 5%. For the 31 August and 26 October collections, isolations from up to ten needle sections per trap plant were attempted and *Phytophthora* cultures identified as outlined in Fraser et al. (2020). Identifications of a subset of *Phytophthora* isolates were also confirmed with species-specific qPCR. For the 5 October and 26 October collections, needle samples were prepared for and analysed by species-specific qPCRs targeting the *Ypt*1 gene region of *P. pluvialis* (McDougal et al. 2021) and *P. kernoviae* (Schena et al. 2006) following the methods outlined in O'Neill et al. (2018).

2018 trial

Trial layout and treatment

The 2018 trial was an extension of the 2017 trial. The control blocks and spray blocks from 2017 were maintained with no additional treatments. Extra blocks were added to both sites to investigate the impact of spray timing. Treatments were planned for November 2017, February 2018 and May 2018. However, due to a misunderstanding with the spray contractor, the February 2018 treatment blocks were inadvertently re-sprayed on 5 May 2018. Extra blocks were added to allow a May only spray treatment, which was carried out on 30 May 2018. Therefore, the five treatments were: unsprayed, 11 February 2017 spray, 24 November 2017 spray, 10 February 2018 and 5 May 2018 sprays, and 30 May 2018 spray. For all treatments there were three replicate blocks at each site, except for the 30 May 2018 treatment, for which there were three blocks at Tram Road but only two blocks at Cashmore's Road.

Disease assessments

Ground-based disease assessments were carried out in September 2018, following the same method as in 2017. In addition to the ground-based scoring, aerial imagery was acquired on 19 October 2018 and used to visually assess disease severity on 50 randomly selected trees per block using GIS (ArcGIS Pro). The imagery was captured using a 4-channel multispectral (NIR+RGB), large-format camera and processed to produce orthorectified images with a 5 cm/pixel resolution. Ground control points were surveyed and marked across the trial area and used to geo-register the imagery. In the field, high-grade GPS points were collected at the corners of each plot and used to create GIS polygons representing the rectangular spatial boundaries of the trial blocks. Trees on the edges of blocks were excluded from analysis by applying a 10 m internal buffer to the plot boundaries. This was done to reduce edge-effects that may have occurred due to overspray or spray drift during aerial fungicide application. Within each plot, 50 locations were randomly selected and the tree nearest to each random point was selected for scoring. Where two points fell on a single tree, the next nearest tree was selected. Where points fell on shadows or other objects in the scene, the nearest tree to the north of the point was selected.

To score the trees in the aerial imagery, RNC was visually assessed by an experienced analyst. Assessments were carried out by evaluating the RGB imagery (Fig. 2), a false-colour composite using the NIR, red and green bands as well as the normalised difference vegetation index (NDVI) defined as:

$$NDVI = (NIR - Red)/(NIR + Red)$$

Using these data, the percentage of discolouration on each crown was estimated visually. The scoring was done by a single analyst and reviewed by an experienced forest pathologist for consistency.

Pathogen identification and inoculum load

Five trap plants were placed in two blocks of each treatment at each site on 21 or 22 June 2018 in the same manner as in 2017. Trap plants were assessed for *Phytophthora* symptoms and symptomatic foliage was sampled on 24 September 2018 and analysed by qPCR, as above.

2019 trial

Trial layout and treatment

The 2019 trial was located at one 26-year-old stand that was adjacent to the Cashmore's Road site. The site was divided into twelve c. 5 ha blocks. Three replicate blocks were each assigned to one of four treatments following a stratified random design: unsprayed control, 7 December 2018 spray, 17 February 2019 spray, and 1 May 2019 spray.

Disease assessments

The method for ground-based disease assessments was slightly modified from previous years, 50 trees were assessed in each block rather than 20, trees were assessed along two transects running through the centre of each block parallel to the cast needle traps (see below) rather than on the road edge, and assessments

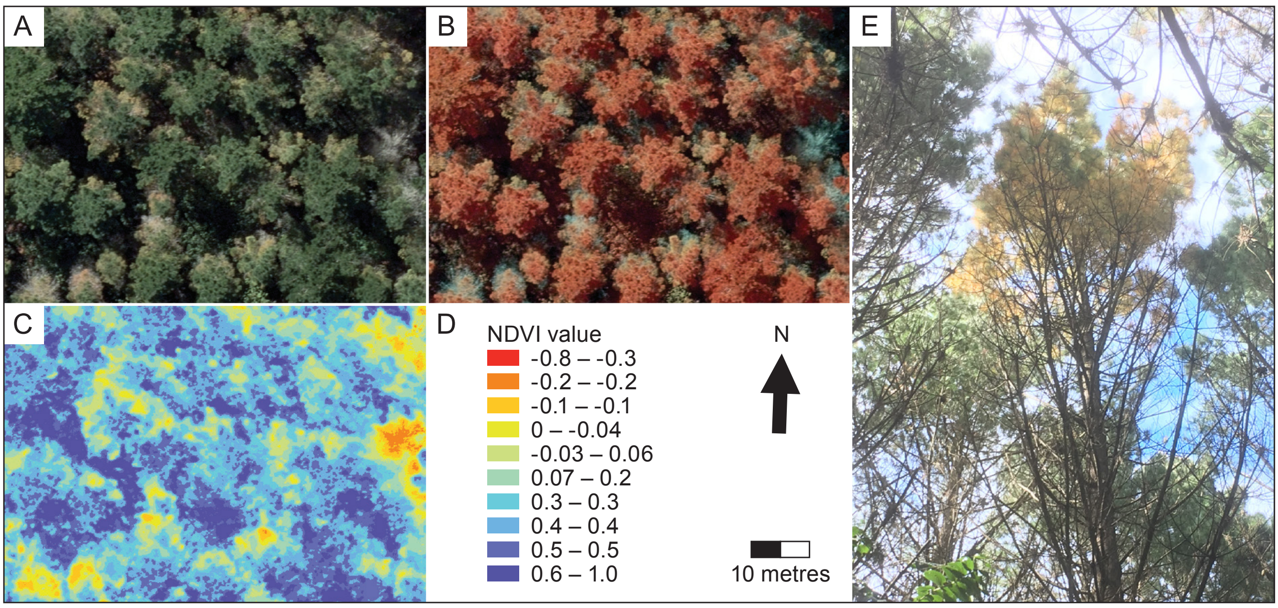

FIGURE 2: Aerial and ground-based imagery of red needle cast affected radiata pine trees in 2018. Aerial imagery: (A) RGB composite, (B) NIR false-colour composite, and (C,D) normalised difference vegetation index (NDVI). Ground-based imagery: (E).

were done twice rather than once. Assessments were undertaken on 29 August 2019 and 6-7 November 2019 due to later development of disease expression that year. The aerial scoring method used for the 2018 trial was repeated in 2019 by the same analyst. The imagery was recaptured using the same platform and sensor. The 2019 orthorectified imagery was geo-registered using the network of existing ground control points established previously.

Quantification of needle cast

Five c. 0.36 m^2 cast needle traps were placed in each block on 20-21 March 2019 following the same layout as in 2017. Understory vegetation was cleared from around each trap to reduce interception of needles and a tarpaulin was placed under each trap to reduce potential contamination from the soil. Cast needles were collected every c. four weeks until 16 December 2019, dried and weighed. At each collection, a subset of still partially green needles was sampled for analysis by species-specific qPCR (as above).

Statistical analyses

Data were analysed using R (R Core Team 2019), supplemented by packages 'plyr' (Wickham 2011) and 'dunn.test' (Dinno 2017). Statistical analyses, including tests for normality (Shapiro-Wilk test; Royston 1982) and homogeneity of variances (Bartlett's test; Bartlett 1937), were accompanied with visual assessments using box and whisker plots, histograms, and QQ plots. To determine whether there were differences in the severity of RNC and needle cast between treatments, the Kruskal-Wallis rank sum test was applied (Hollander & Wolfe 1973). This non-parametric test was used in favour of parametric models and ANOVA analysis, as recommended by Lantz (2013), because sample distributions were not normally distributed. Even when transformed using the usual transformation functions (i.e. logarithmic, logit, arcsine, and square root transformations), tests for both normality (Shapiro-Wilk test; Royston 1982) and homogeneity of variances (Bartlett's test; Bartlett 1937) failed. Therefore, the Kruskal-Wallis test, which makes no assumptions about the underlying distributions was appropriate. When the Kruskal-Wallis rank sum test indicated significant differences (α = 0.05), post-hoc testing via the Dunn test (Dunn 1961, 1964) using a Bonferroni adjustment (Haynes 2013) to control the familywise error rate, was used to determine where those differences occurred; i.e. which levels of the independent variable differed from each other level. For consistency, the 'altp' option within 'dunn.test' (Dinno 2017) which provides p-values that are significant if less than the α-value, was invoked. We note, however, that p-values represent only an index to evidence (Burnham & Anderson 2014) and do not imply practical significance (Murtaugh 2014). Therefore, we also consider cases for which α exceeds 0.05 but is less than 0.10.

For 2017 trap tree data, disease severity was analysed with linear mixed models (LMMs) fitted by restricted maximum likelihood (REML) (R package lme4, Bates et al. 2015), after arcsine transforming severity scores. Disease incidence and detection of *Phytophthora* by qPCR was analysed independently with generalised linear mixed models (GLMMs) assuming a binomial distribution and logit link. Counts of isolates of the two species of *Phytophthora* from the trap plants were analysed separately with negative binomial GLMMs using a log link (due to overdispersion). For all mixed-effects models, treatment (factor with two levels: sprayed or unsprayed control), site (factor with two levels: Cashmore's Road and Tram Road) and their interaction were included as fixed effects and 'trap tree' nested in 'block' in 'site' was modelled as random effects. The GLMMs were fitted using Laplace approximation (R package glmmADMB, Skaug et al. 2016). For model validation, plots of Pearson residuals against the fitted values and versus each explanatory variable in the model, and residual diagnostics based on a simulation-based approach (R package DHARMa, Hartig 2021), were used. In all cases, model selection for the fixed effect terms was based on Akaike's Information Criterion (AIC) and likelihood ratio tests (Zuur et al. 2013).

Results

2017 trial

Ground-based assessments

When 2017 assessment data from both sites were pooled, RNC severity was significantly lower (p = 0.002) in sprayed blocks (mean 5.5%, sd 11.6) than in unsprayed control blocks (mean 10.9%, sd 15.2) (Fig. 3). At Tram Road, RNC severity was significantly lower (p = 0.005) in sprayed blocks (mean 4.6%, sd 8.7) than unsprayed blocks (mean 10.9%, sd 14.7). However, at Cashmore's Road, RNC severity was only marginally significantly lower (p = 0.096) in sprayed blocks (mean 6.5%, sd 14.0) than unsprayed blocks (mean 10.8 %, sd 15.8).

Cast needle quantification

Overall, pine needle cast in 2017 was lower in sprayed blocks (mean 0.92 g m^{-2} day^{-1}, sd 0.57) than in unsprayed control blocks (mean 1.15 g m^{-2} day^{-1}, sd 0.74) (Fig. 4). However, the difference was not significant when data were pooled from both sites (p = 0.111). Analyses of needle cast at the Tram Road site indicated significant differences between treatments (p = 0.019) with needle cast in the sprayed blocks (mean 0.98 g m^{-2} day^{-1}, sd 0.66) being significantly lower than in the unsprayed control blocks (mean 1.43 g m^{-2} day^{-1}, sd 0.88). At Cashmore's Road, differences between sprayed (mean 0.90 g m^{-2} day^{-1}, sd 0.49) and unsprayed (mean 0.92 g m^{-2} day^{-1}, sd 0.50) treatments were not significant (p = 0.952).

Pathogen identification in cast needles

Both *P. pluvialis* and *P. kernoviae* were detected by qPCR from cast needle samples collected on 14 September 2017 (Table S1). *Phytophthora pluvialis* was detected from 17% of samples, while *P. kernoviae* was detected from 25% of samples. Treatment did not have a significant impact on the probability detection of either pathogen (p > 0.05).

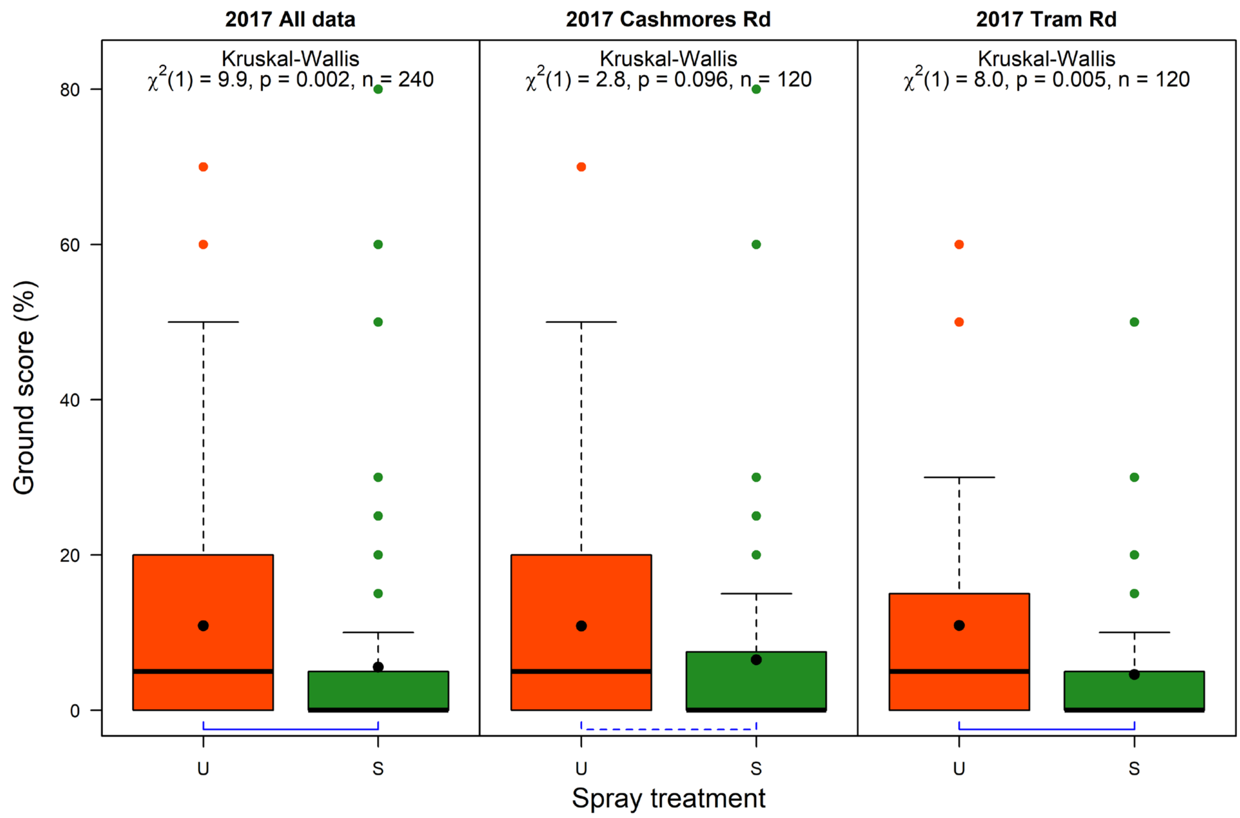

FIGURE 3: Impact of low-volume aerial application of cuprous oxide on ground-based red needle cast severity scores in 2017. Spray treatments: U, unsprayed control; S, sprayed. Significant differences (Dunn test) are indicated as a blue solid line (α = 0.05) or dashed line (α = 0.10).

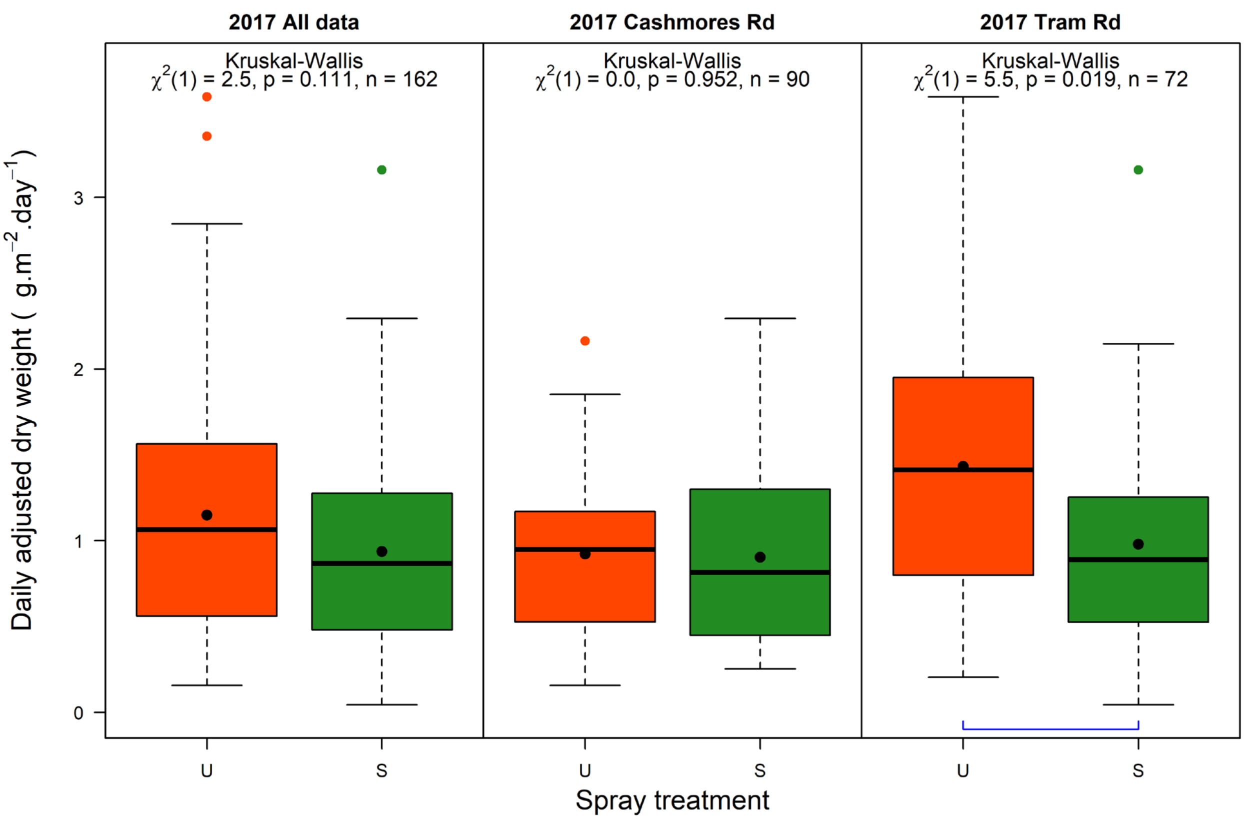

FIGURE 4: Impact of low-volume aerial application of cuprous oxide on pine needle cast in 2017. U, unsprayed control; S, sprayed control. Significant differences (Dunn test, α = 0.05) are indicated by blue lines.

Pathogen identification and disease development on trap plants

Both *P. pluvialis* and *P. kernoviae* were also detected from the trap plants (Table S2). Across all sampling time points and both detection methods, *P. pluvialis* was detected from 43% of samples and *P. kernoviae* from 62% of samples.

Symptoms of *Phytophthora* infection, including olive lesions and typical black bands, were first observed on trap plants at the end of August, after one month in the field (Table S3). The numbers of needles on each plant displaying symptoms at this point was low and severity was not scored. Incidence of symptoms was significantly greater (p = 0.002) in unsprayed (80% of trees) than sprayed blocks (44% of trees). Both *P. pluvialis* and *P. kernoviae* were isolated from samples collected at both sites at this time (Fig. S1). The total number of isolates of *P. pluvialis* was significantly greater (p = 0.023) from samples collected in unsprayed (44 isolates) than sprayed blocks (4 isolates). A similar trend was seen for isolates of *P. kernoviae*, but the difference was not statistically significant (91 isolates from unsprayed blocks; 45 isolates from sprayed blocks; p > 0.05).

By 5 October, after two months in the field, the severity of symptoms on trap plants had progressed rapidly (mean 86%, sd 24). Most fully developed needles had a grey/white appearance. Incidence and severity were scored at this point; treatment, site and their interaction did not have a significant impact on either disease measure (p > 0.05). Both *P. pluvialis* and *P. kernoviae* were detected by qPCR at this point. There was a significant interaction between site and treatment for the detection of *P. pluvialis* (p = 0.034), with greater detection in unsprayed blocks at Tram Road, but greater detection in sprayed blocks at Cashmore's Road. Treatment, site or the interaction between the two were not significant for the detection of *P. kernoviae*.

By 26 October, after almost three months in the field, most of the fully developed needles were dead (seemingly resulting from *Phytophthora* infection) on the trap plants under both treatments. Typical early symptoms of *Phytophthora* infection, olive lesions and black bands, were observed on some developing needles. The total number of isolates of *P. kernoviae* was significantly greater (p = 0.049) from samples collected in sprayed (38 isolates) than unsprayed blocks (19 isolates). However, treatment did not have a significant impact (p > 0.05) on the total number of *P. pluvialis* isolates at this time (40 isolates from unsprayed blocks; 41 from sprayed blocks). Treatment, site and the interaction between the two were not significant for the detection of either pathogen by qPCR (p > 0.05).

As both isolation and qPCR were used for detection of *Phytophthora* at the end of October, the sensitivity of the two methods could be compared (Table S4). Detection of *P. pluvialis* did not differ significantly between the two methods (p > 0.05; qPCR, 53%; isolations, 46%); however, detection of *P. kernoviae* was significantly higher by qPCR than isolation (p = 0.009; qPCR, 69%; isolations, 48%). Detection of *P. pluvialis* and/or *P. kernoviae* was significantly higher by qPCR than isolation (p < 0.001; qPCR, 97%; isolations, 75%).

2018 trial

Ground-based assessments

When 2018 data from both sites were pooled and analysed together, and different treatments were grouped into sprayed (November 2017, February and May 2018, May 2018) or unsprayed (control and sprayed February 2017), RNC severity was significantly lower (p = 0.011; Fig. 5) in sprayed blocks (mean 6.8%, sd 16.6) than in unsprayed blocks (mean 9.5% ± 18.2 sd). This result was largely influenced by significant differences at Cashmore's Road (p < 0.001), as differences between treatments at Tram Road, although showing a similar trend, were not significant (p = 0.695).

There were also significant differences among the five individual treatments at Cashmore's Road (p < 0.001; Fig. 5) but not at Tram Road (p = 0.10; Fig. 5). At Cashmore's Road, RNC severity in the May spray blocks (mean 2.5%, sd 14.2) was significantly lower (p < 0.01) than in both the unsprayed control blocks (mean 10.3%, sd 19.4) and the February 2017 spray blocks (mean 10.5%, sd 18.7).

Aerial assessments

Similar to ground assessment results, when 2018 aerial data from both sites were pooled, and treatments grouped into sprayed or unsprayed, RNC severity was significantly lower (p < 0.001; Fig. 6) in the sprayed blocks (mean 4.4 %, 15.2 sd) than in the unsprayed blocks (mean 11.8 %, sd ± 26.1). As with the ground scores, this result was largely influenced by significant differences at Cashmore's Road (p < 0.001), as differences between treatments at Tram Road were not significant (p = 0.1).

There were also significant differences among the five individual treatments at Cashmore's Road (p < 0.001; Fig. 6) but not at Tram Road (p = 0.120; Fig. 6). At Cashmore's Road, RNC severity in the November (mean 5.5%, sd 17.0), February and May (mean 7.3%, sd 18.2), and May (mean 2.5%, sd 10.5) spray blocks was significantly lower (p < 0.005) than in the unsprayed control blocks (mean 18.8%, sd 32.4). RNC severity was also significantly lower (p < 0.010) in the May spray blocks than in the February 2017 spray blocks (mean 14.2%, sd 28.1).

Pathogen identification on trap plants

Across both sites and all treatments, *P. pluvialis* was detected from 13% of trap plants and *P. kernoviae* from 15% of trap plants. Generally, detection of both species was greater from trap plants in unsprayed blocks (20%, *P. pluvialis*; 20% *P. kernoviae*) than sprayed blocks (7%, *P. pluvialis*; 11% *P. kernoviae*). Symptom severity was also greater on trap plants in unsprayed blocks (mean 31%, sd 30) than in sprayed blocks (mean 14%, sd 25).

2019 trial

Ground-based assessments

The 2019 trial was assessed twice from the ground, first in August 2019 and again in November 2019, due to later disease development compared with previous years. Disease severity was very low in August and no significant differences between treatments were

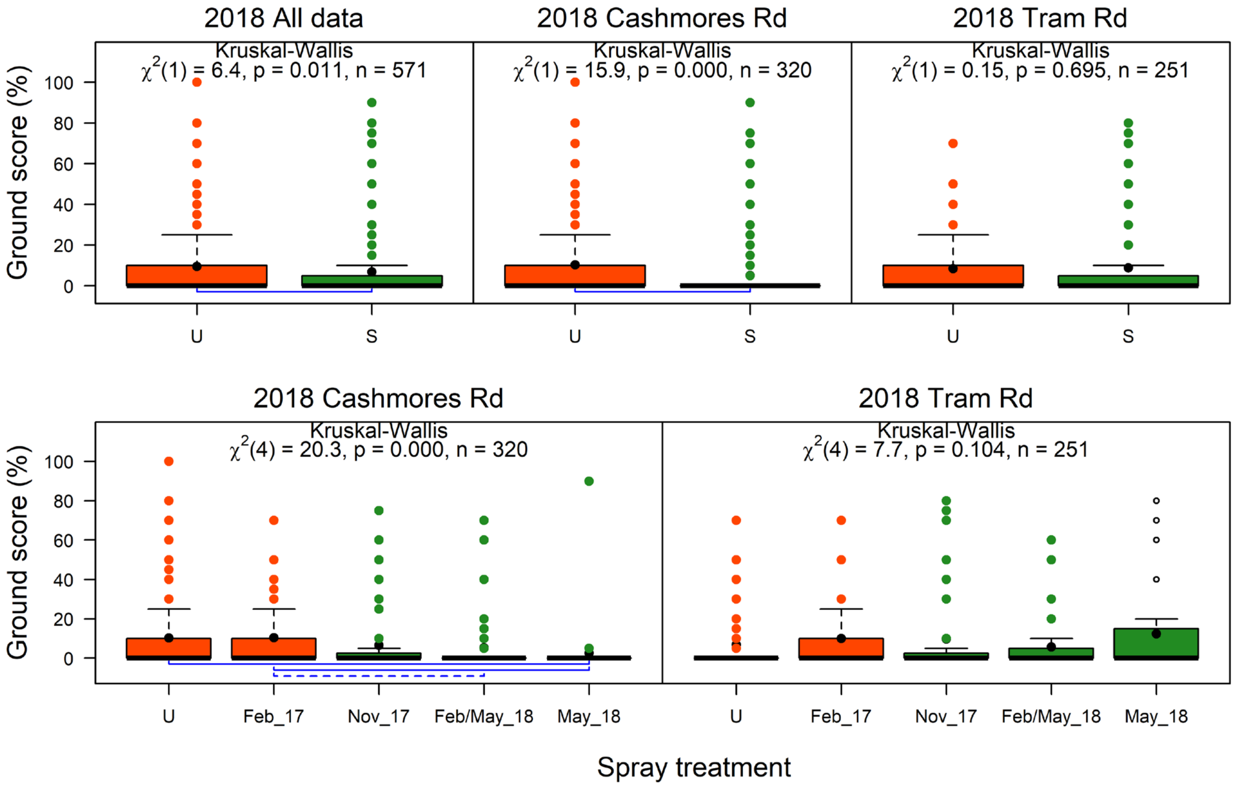

FIGURE 5: Impact of low-volume aerial application of cuprous oxide on ground-based red needle cast severity scores in 2018. In the top row, treatments have been grouped into those involving a spray application (S, sprayed), and those with no spray within the period of the trial (U, unsprayed). Significant differences between treatment pairs (Dunn test) are indicated as a blue solid line (α = 0.05) or dashed line (α = 0.10).

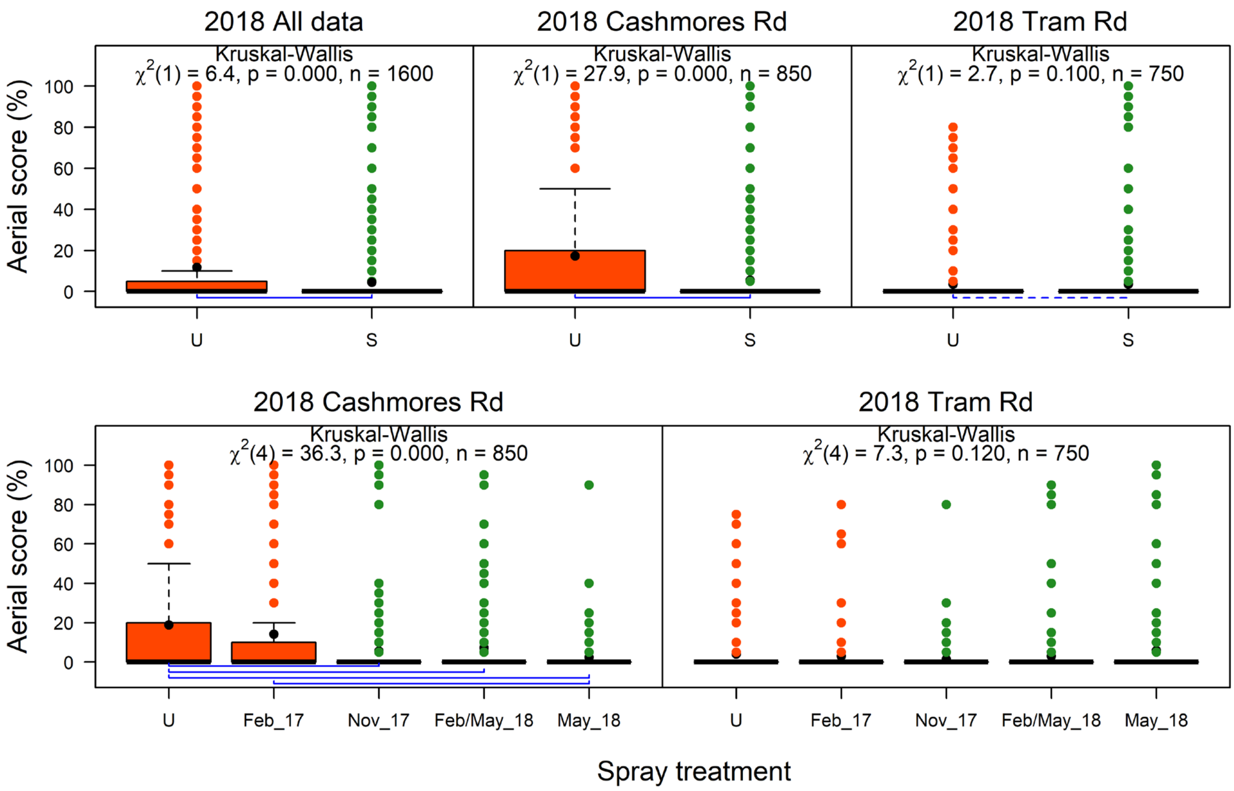

FIGURE 6: Impact of low-volume aerial application of cuprous oxide on red needle cast severity scores from aerial imagery in 2018. In the top row, treatments have been grouped into those involving a spray application (S, sprayed), and those with no spray within the period of the trial (U, unsprayed). Significant differences between treatment pairs (Dunn test) are indicated as a blue solid line (α = 0.05) or dashed line (α = 0.10).

observed (Fig. 7). However, significant differences between treatments were observed for the November assessments (p = 0.001). When the different spray treatments were pooled, RNC severity in November was significantly lower (p = 0.001) in the sprayed blocks (mean 12.9 %, sd 19.1) than in the unsprayed blocks (mean 21.1%, sd 25.3). When the four spray treatments were analysed separately, RNC severity in November was significantly lower (p < 0.05) under the November (mean 12.3%, sd 17.9) and February (mean 11.5%, sd 18.6) spray blocks than in the unsprayed control blocks (mean 21.1%, sd 25.3). RNC severity in the April spray blocks (mean 14.9%, sd 20.7) did not differ significantly from the other treatments.

Aerial assessments

Very similar results were seen for the aerial assessment data. When 2019 aerial assessment data were analysed with the spray treatments grouped together, RNC severity was significantly lower (p = 0.006; Fig. 8) in the sprayed blocks (mean 9.0%, sd 17.0) compared to the unsprayed blocks (mean 16.0%, sd 25.0). Significant differences (p = 0.001) were also found among the four individual treatments. RNC severity was significantly lower (p < 0.05) under both the November (mean 7.0%, sd 15.0) and February spray treatments (mean 7.0%, sd 16.0) compared to the control (mean 16.0%, sd 25.0). As with the ground assessments, RNC severity in the April spray blocks did not differ significantly from the other treatments (p > 0.05).

Cast needle quantification

When 2019 needle cast data were analysed with the spray treatments grouped together, needle cast was significantly lower (p < 0.001; Fig. 9) in sprayed blocks (mean 0.22 g day^{-1}, sd 0.12) compared to the unsprayed control blocks (mean 0.29 g day^{-1}, sd 0.16). There were also significant differences (p < 0.001) among the four individual treatments. Needle cast was significantly lower (p < 0.05) under all spray treatments than the unsprayed control treatment. Needle cast was significantly lower (p = 0.02) under the April treatment (mean 0.19 g day^{-1}, sd 0.11) than the November (mean 0.23 g day^{-1}, sd 0.12). Needle cast in the April treatment was also marginally significantly lower than in the February treatment (mean 0.23 g day^{-1}, sd 0.11).

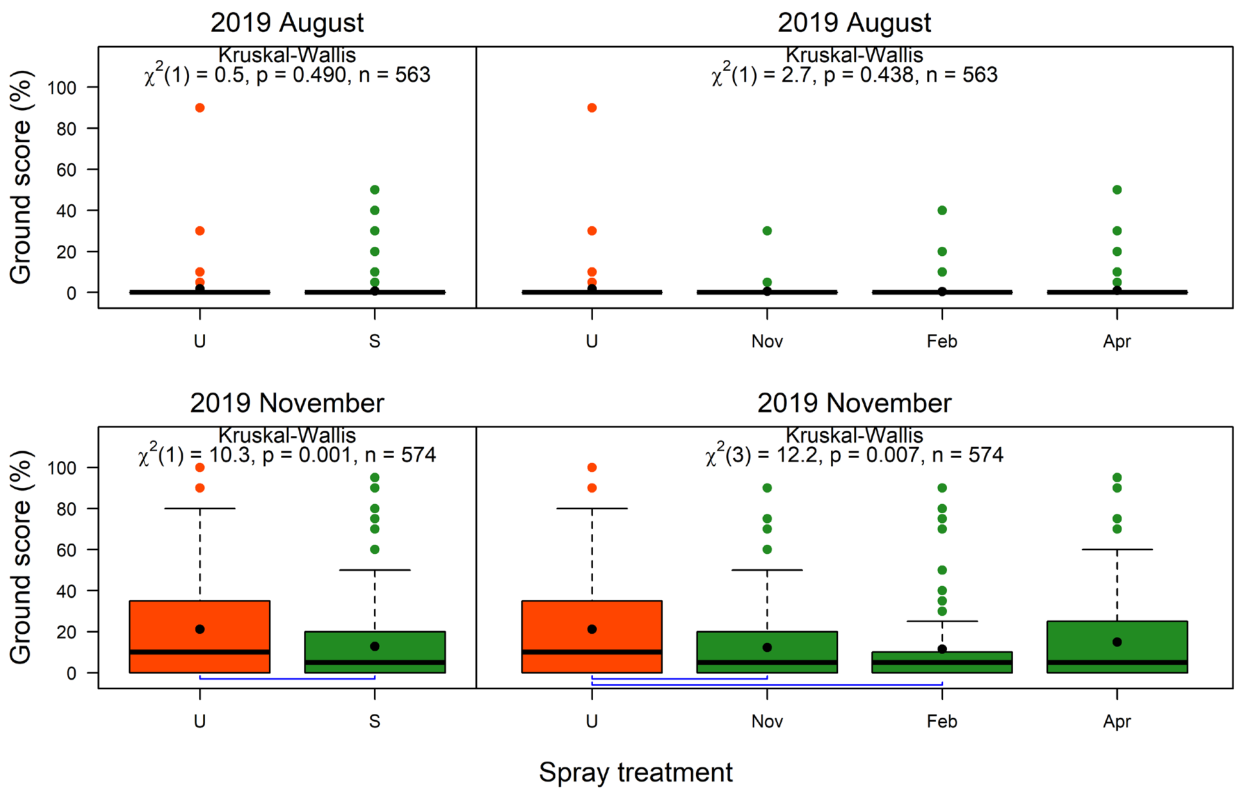

FIGURE 7: Impact of low-volume aerial application of cuprous oxide on ground-based red needle cast severity scores in 2019. Ground-based assessments were undertaken at two timepoints, in August 2019 (top) and November 2019 (bottom). In the left column, treatments have been grouped into those involving a spray application (S, sprayed), and those with no spray within the period of the trial (U, unsprayed). Significant differences between treatment pairs (Dunn test) are indicated as a blue solid line (α = 0.05).

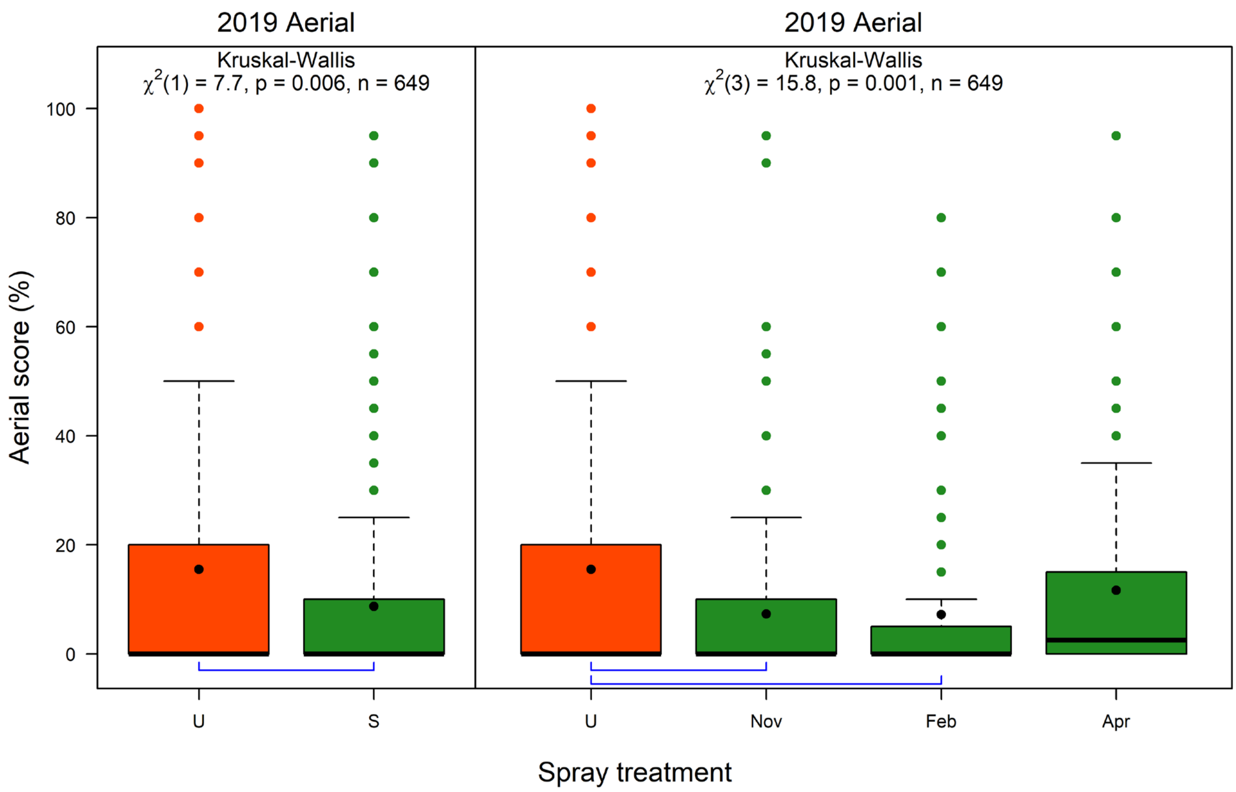

FIGURE 8: Impact of low-volume aerial application of cuprous oxide on red needle cast severity scores from aerial imagery in 2019. In the left graph, treatments have been grouped into those involving a spray application (S, sprayed), and those with no spray within the period of the trial (U, unsprayed). Significant differences between treatment pairs (Dunn test) are indicated as a blue solid line (α = 0.05).

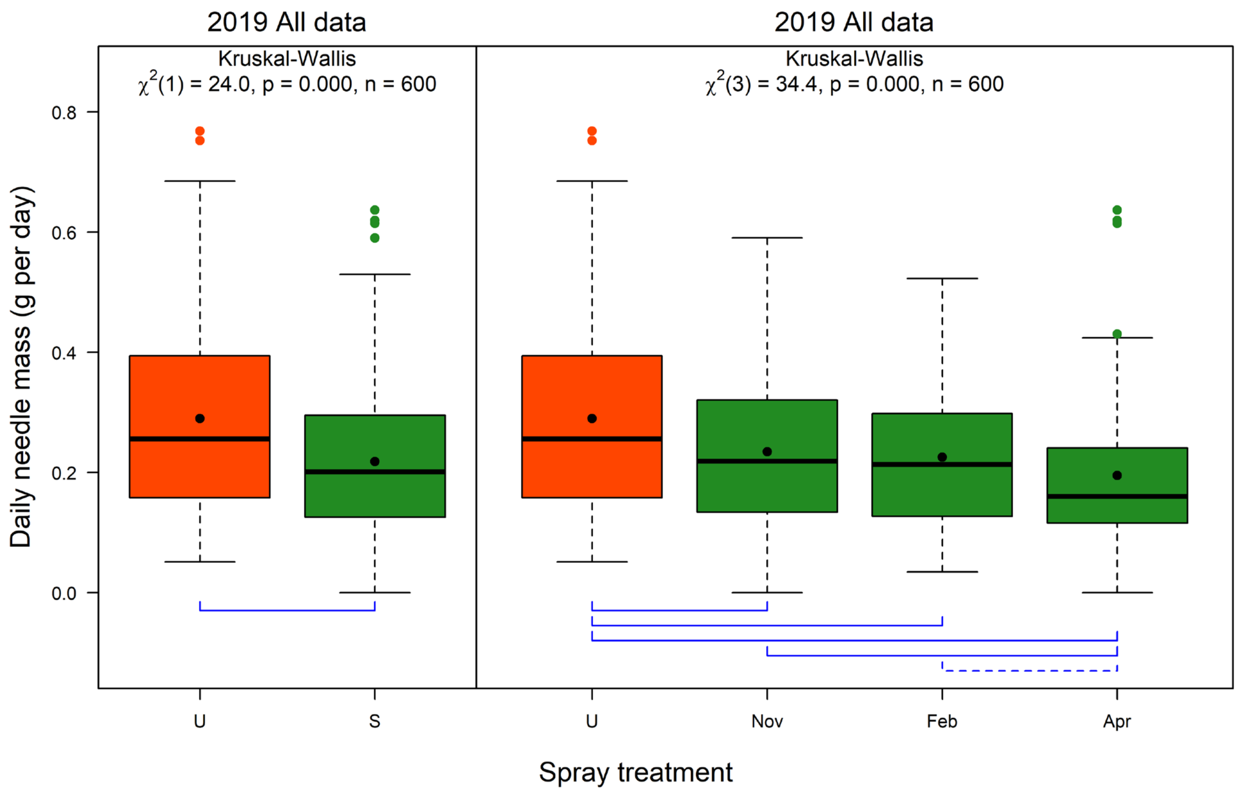

FIGURE 9: Impact of low-volume aerial application of cuprous oxide application on needle cast in 2019. In the left graph, treatments have been grouped into those involving a spray application (S, sprayed), and those with no spray (U, unsprayed). Significant differences between treatment pairs (Dunn test) are indicated as a blue solid line (α = 0.05) or dashed line (α = 0.10).

Pathogen identification from cast needles
Both *P. pluvialis* and *P. kernoviae* were detected from only 1% of cast needle samples in the 2019 trial. Positive samples were collected in early July, early and late August, and early November. Most positive samples were collected from the November treatment blocks (3% positive for both species). No *P. pluvialis* was detected from the February treatment blocks, but 1% of samples from these blocks were positive for *P. kernoviae*. No samples from the April blocks were positive for either pathogen. Both *P. pluvialis* and *P. kernoviae* were detected from 1% of samples from unsprayed control blocks.

Discussion

These results are the first to confirm that low-volume aerial applications of cuprous oxide can reduce the severity of red needle cast, caused by *Phytophthora pluvialis* and *P. kernoviae*, in commercial radiata pine plantations. Cuprous oxide applied at 0.855 kg ha^{-1} active ingredient, the standard dose currently used to control Dothistroma needle blight, significantly reduced RNC severity in all three trials undertaken between 2017 and 2019. As well as reducing disease severity, application of copper also tended to reduce needle cast and infection on trap plants in years when these were also assessed. These trials were also the first to investigate optimal spray timing for control of RNC, incorporating November, February and April (or May) treatments into the experimental design. However, no consistent effect of spray timing was observed. Generally, all three spray timings reduced disease severity compared to the unsprayed control, but differences were not always statistically significant, and few differences were detected between different spray timings. These findings are highly relevant for the development of methods to manage RNC.

The results of this work agree with previous controlled *in vitro* and *in planta* studies that showed that copper fungicides inhibited the growth and sporulation of, and infection by, *P. pluvialis* and *P. kernoviae* (Rolando et al. 2017; Rolando et al. 2019). As outlined in Rolando et al. (2019), copper likely acts on *P. pluvialis* and *P. kernoviae* in much the same way as it acts against *Dothistroma septosporum*. Copper ions slowly dissolve in water and are distributed across treated needle surfaces (Franich 1988). These ions will inhibit spore production and kill spores that spread to treated needles (Rolando et al. 2017). Under normal conditions without copper, transient hyphae of *P. pluvialis* emerge from stomata during wet periods and produce semi-caducous sporangia, which produce motile zoospores that are thought to disperse in water films and water splash and initiate new infections. It is likely that copper ions inhibit the production of transient hyphae and sporangia and kill any free zoospores, reducing inoculum levels and resulting infection (Rolando et al. 2017; Rolando et al. 2019).

Despite *P. pluvialis* being the more common cause of RNC nationally (Dick et al. 2014; Fraser et al. 2020), both *Phytophthora* species co-occur at certain sites (Fraser et al. 2020; Hood et al. 2022), and *P. kernoviae* is found independently at others (Fraser et al. 2020). Both species were detected at the trial sites, and, at times, *P. kernoviae* seemed to be more abundant. This is a similar pattern to that seen in the same forest by Hood et al. (2022). *Phytophthora kernoviae* has a long history in the forest where the trials took place, having been first isolated from there in the 1950s (McAlonan 1970; Ramsfield et al. 2007). Both *P. pluvialis* and *P. kernoviae* seem to have a similar sensitivity to copper (Rolando et al. 2017) and similar epidemic behaviour (Fraser et al. 2020; Hood et al. 2022) and we may expect copper to have a similar operational efficacy against both. However, these trials should be replicated in areas where *P. pluvialis* is more dominant, such as in forests on the east coast of the North Island (Fraser et al. 2020), to provide greater confidence in the generalisability of our results. This region is prone to more frequent severe RNC outbreaks, so repeating trials there will also increase the probability of testing copper under greater disease pressure.

The results of this study did not support the hypothesis that copper applications in autumn (March-May) would be most effective for control of RNC (Fraser et al. 2020; Hood et al. 2022; Rolando et al. 2019). In the 2018 trial, there was significantly reduced RNC under all three spray treatment timings (November only, February and May, and May only) compared to unsprayed controls at one site. However, no differences were detected between treatments at the other site where disease levels were lower. At the site where copper applications reduced disease, there were no differences in RNC severity between the spray timing treatments. In the 2019 trial, RNC severity was significantly lower under both the November and February spray treatments compared to the unsprayed control, but there was no difference between the April treatment and any other treatment, including the unsprayed control. This may mean that reducing inoculum early in the epidemic is more important than protecting needles later in the season, when inoculum loads tend to peak (Fraser et al. 2020). Alternatively, this finding may indicate that *P. pluvialis* and *P. kernoviae* are more sensitive to copper than *D. septosporum*, and the residues remaining on the needles after 3 or more months are still enough to inhibit or kill both species of *Phytophthora*. Copper persistence has been shown to be about 3 months (Gilmour & Noorderhaven 1973; Rolando et al. 2017; Rolando et al. 2019), after which copper residues are reduced by 70-80%. However, it is important to note that overall disease levels were quite low in the trials, reflecting generally low levels of disease in the region in 2018 and 2019. Therefore, these results should be considered with caution and no strong conclusions can be made. Further trials are required to test optimal treatment timing, as well as overall copper efficacy, under higher disease pressure.

Nonetheless, the finding that cuprous oxide applied at the industry standard rate for DNB also, in many cases, will reduce RNC severity is an important development for the New Zealand forestry industry. Prior to the present study, no control options were available to protect plantations from RNC. However, there is a well-developed programme for the control of DNB in New Zealand, coordinated by the New Zealand Forest Owner's Association Dothistroma Control Committee (DCC), which is now in its 56th year. For greater efficiency, the DCC buys cuprous oxide and oil in bulk and organises aerial spray contractors. As outlined in the introduction, disease assessments are undertaken in winter (June and July) and copper is applied in late spring (November) for control of DNB, with a second spray in late summer (February) if levels justify. That November and February spray applications appear to also be effective against RNC could be convenient for control of this disease. However, the rapid development of RNC symptoms within a year and typical lack of repeated severe epidemics at a particular site year-on-year (Dick et al. 2014; Fraser et al. 2020) will make design of a targeted spraying regime difficult. DNB tends to build-up over years, with carry-over of inoculum from one year to the next being important, allowing informed management decisions based on both previous and current year disease levels. In contrast, heavy RNC defoliation events tend not to re-occur year on year on at a particular site, and RNC epidemics may develop on trees that appear green and healthy in Autumn (March or April), but then become severely defoliated by the following spring (Dick et al. 2014). A more reactive control programme may be necessary for RNC, with more frequent surveys from March onwards and rapid responses if symptoms appear. A priority for future research must be to understand the drivers behind annual variation in disease expression and determine the importance of prior or current disease levels in predicting future disease, with an aim to forecast when an epidemic will occur.

Due to its aquatic toxicity, cuprous oxide is designated as a restricted highly hazardous pesticide by the Forest Stewardship Council (FSC 2019a, 2019b). However, the low-volume applications undertaken for DNB, which were shown to be effective for RNC here, have been demonstrated to pose a low risk to the receiving aquatic environments in the Central North Island of New Zealand (Baillie et al. 2017). Further research should be undertaken to fully assess the risk to aquatic environments if additional operational sprays are to be undertaken for RNC, particularly as these treatments may target stands over 15 years old (beyond the age of treatment for DNB), different seasons, and new regions. Likewise, an investigation into the impact of additional sprays on beneficial foliar and rhizosphere microbial communities is warranted.

Conclusions

The results reported here are the first to show that low-volume aerial applications of cuprous oxide applied at 0.855 kg ha^{-1} active ingredient can reduce the severity of red needle cast, caused by *Phytophthora pluvialis* and *P. kernoviae*, in commercial radiata pine plantations. No consistent effect of spray timing (November, February and April (or May)) was detected. However, disease levels were relatively low in years when spray timing was tested, and further trials are required to test this aspect under greater disease pressure. Another priority for future research must be to understand the drivers behind annual variation in disease expression. The importance of prior disease levels, combined with prior and future weather conditions, in predicting disease must be determined, with an aim to develop disease forecasts. These findings support the management of RNC in radiata pine plantations.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

Project conception: SF, MB, LB, CR
Experimental design: SF, MB, IH, LB, CR
Coordination of copper treatments: MB
Remote sensing lead: GP
Acquisition of data: SF, HJE, IH
Analysis and interpretation of data: CT, CS, SF
Drafting of the manuscript: SF, MB, GP, CT, CS, HJE, IH, LB, CR

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Supplementary Tables and Figures

TABLE S1: Impact of low-volume aerial application of cuprous oxide on the detection of species of *Phytophthora* by qPCR in needle cast in 2017.

Site/treatment	Detection (% of samples positive)	
	P. pluvialis	***P. kernoviae*** [a]
Cashmore's Rd		
Control	11	0
Spray	11	22
Tram Rd		
Control	22	44
Spray	22	33

[a] Detections of *P. kernoviae* were significantly greater at Tram Rd than Cashmore's Rd

TABLE S2: Impact of low-volume aerial application of cuprous oxide on the detection of species of *Phytophthora* by isolation on trap trees in 2017.

Site/treatment	Mean number of isolates recovered from trap trees			
	P. pluvialis		***P. kernoviae***	
	31 August	**26 October**	**31 August**	**26 October**
Cashmore's Rd				
Control	0.9 ± 0.5 A	1.5 ± 0.5 A	2.6 ± 0.9 A	0.7 ± 0.2 A
Spray	0.1 ± 0.1 B	1.5 ± 0.4 A	1.1 ± 0.4 A	1.1 ± 0.4 B
Tram Rd				
Control	1.5 ± 0.6 A	0.7 ± 0.3 B	2.4 ± 0.5 A	0.3 ± 0.1 A
Spray	0.2 ± 0.1 B	0.8 ± 0.4 B	1.4 ± 0.5 A	1.1 ± 0.4 B

Treatments followed by different letters differ significantly (within a column) ($p < 0.05$)

TABLE S3: Impact of low-volume aerial application of cuprous oxide on symptoms of *Phytophthora* needle diseases on trap trees in 2017.

Site/treatment	Mean trap tree disease scores			
	31 August		**5 October**	
	Incidence[a]	**Severity**[b]	**Incidence**	**Severity**
Cashmore's Rd				
Control	72 ± 11 A		100 ± 0 A	93.8 ± 2.5 A
Spray	39 ± 12 B		94 ± 6 A	85.0 ± 5.5 A
Tram Rd				
Control	89 ± 8 A		94 ± 6 A	85.9 ± 5.7 A
Spray	50 ± 12 B		94 ± 6 A	78.8 ± 8.2 A

Treatments followed by different letters differ significantly (within a column)
[a] Highly significant, $p < 0.01$; % trees with symptoms
[b] % needles with symptoms

TABLE S4: Impact of low-volume aerial application of cuprous oxide on the detection of species of *Phytophthora* by qPCR on trap trees in 2017.

Site/treatment	Detection (% of samples positive)			
	P. pluvialis		*P. kernoviae*	
	5 October	26 October	5 October	26 October
Cashmore's Rd				
Control	56	61 (44)[a]	72	67 (50)[a]
Spray	70	53 (59)	100	71 (53)
Tram Rd				
Control	65	53 (47)	82	59 (35)
Spray	29	43 (35)	82	81 (53)

[a] Values in brackets show the isolation success (%) from the same needle samples

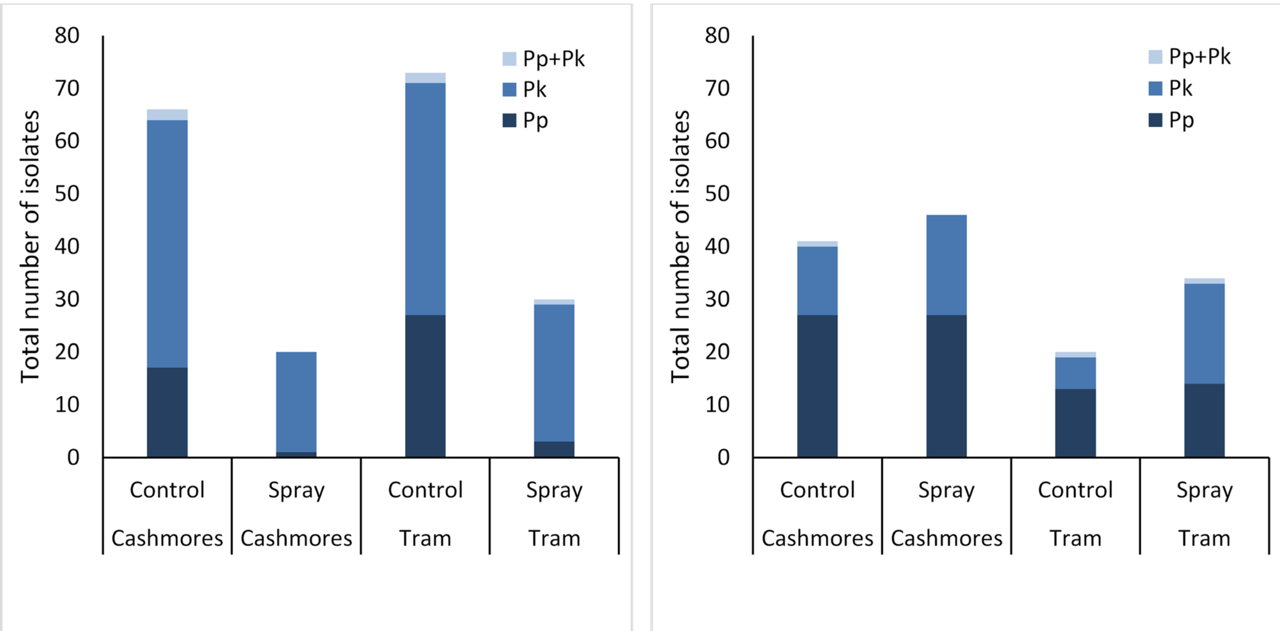

FIGURE S1: Impact of low-volume aerial application of cuprous oxide on number of isolates of *Phytophthora* from trap trees in 2017. Left, 31 August isolates; right, 26 October isolates. Pp, *P. pluvialis*; Pk, *P. kernoviae*; Pp+Pk, mixed cultures.