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Infection periods of *Phytophthora pluvialis* and *Phytophthora kernoviae* in relation to weather variables and season in *Pinus radiata* forests in New Zealand

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Abstract

Background: Red needle cast caused by *Phytophthora pluvialis* Reeser, Sutton & E. Hansen, and less frequently *P. kernoviae* Brasier, Beales & S.A.Kirk, is an important foliar disease of *Pinus radiata* D.Don (radiata pine) in plantations throughout parts of New Zealand. Significant growth loss occurs following years when severe outbreaks occur. Aerial spraying with a copper-based fungicide has potential for disease control. Research is being carried out to optimise application timing, supported by complementary studies to understand RNC epidemiology.

Methods: In order to determine the pathogen infection periods, a field trial was conducted over two years at two forests in the Central North Island of New Zealand. Batches of potted radiata pine seedlings were placed beneath diseased pine stands at fortnightly intervals, before returning them to an open nursery area for assessments of infection every two weeks (based on visual symptoms and qPCR) over a period of three months. A hybrid modelling approach was employed to establish relationships between the proportion of plants showing symptoms and weather conditions during the fortnight of exposure and previous fortnights. Gradient boosting machine learning analyses were used to identify the most important weather variables, followed by analysis of these by generalised mixed effects models, generalised least square models and ordinary least square models.

Results: Development of RNC symptoms and detection of *Phytophthora pluvialis* and *P. kernoviae* on exchange seedlings was greatest for those exposed between April and September (Southern Hemisphere mid-autumn to early-spring). At this time, temperatures, solar radiation and evapotranspiration were lower, and rainfall and foliage wetness were plentiful. Modelling identified temperature and relative humidity several months before the date of exposure as the most important weather variables explaining infection.

Conclusions: Because of autocorrelation, it was not possible to determine those variables that drive sporulation, dispersal, infection and symptom development. This will require more detailed exchange plant studies together with controlled environment inoculation experiments. Nevertheless, results of this and earlier work complement recent research indicating that it may be possible to manage RNC by fungicide applications made in late summer or autumn, early in the annual disease cycle.

Keywords: epidemiology, infection period, needle disease, *Phytophthora kernoviae, Phytophthora pluvialis*, *Pinus radiata*, red needle cast, seedlings, weather variables

Introduction

Red needle cast (RNC) is a foliar disease of *Pinus radiata* D.Don (radiata pine) and *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) in New Zealand caused by the oomycete *Phytophthora pluvialis* Reeser, W. Sutton & E.M.Hansen (Dick et al. 2014; Hansen et al. 2015). *Phytophthora pluvialis* is also responsible for needle loss and twig symptoms on Douglas-fir and twig and stem cankers on *Notholithocarpus densiflorus* (Hook. & Arn.) Manos, C.H.Cannon & S. Oh (tanoak) in Oregon (Reeser et al. 2013; Hansen et al. 2015). Recently it was reported causing cankers on *Tsuga heterophylla* (Raf.) Sarg. (western hemlock) in the United Kingdom (Pérez-Sierra et al. 2022). In New Zealand, the pathogen was first detected in the eastern North Island in 2008 (Dick et al. 2014) and is now found throughout the country (Graham et al. 2018). Outbreaks of RNC have been intermittent and uneven, varying in severity in different years, with greater prevalence in certain regions such as the eastern North Island (Dick et al. 2014; Ganley et al. 2014). The disease is also expressed seasonally, and from late autumn, through winter and into spring, crowns on diseased trees change gradually from green through red-brown to brown, defoliate and concurrently re-green with the development of the new season's flush. These changes in the expression of RNC begin at the base of the crown and progress upwards. Growth increment is significantly reduced in the year following a severe disease event (P.N. Beets, pers. comm.). *Phytophthora kernoviae* Brasier, Beales & S.A.Kirk is also, to a lesser extent than *P. pluvialis*, isolated from foliage on radiata pine trees affected by RNC in New Zealand (Dick et al. 2014). Both *Phytophthora* species produce indistinguishable short, discrete, olive or khaki coloured lesions marked with tiny black specks or bands that contrast with the fresh green colour of the remaining healthy needle tissue.

Chemical control studies have shown that a copper fungicide used routinely to treat dothistroma needle blight, caused by the ascomycete *Dothistroma septosporum* (Dorogin) M.Morelet, in New Zealand radiata pine plantations can also be effective against RNC under controlled conditions (Rolando et al. 2014, 2017, 2019) and in plantations (Fraser et al. 2022). Research is proceeding towards the development of recommendations for operational aerial spray applications (Fraser et al. 2022). In order to assist this work, detailed knowledge of the epidemiology of both *Phytophthora* pathogens is needed (Hood et al. 2017).

Significant epidemiological work has already been initiated. A prototype dynamic systems model has been developed as a basis for understanding the behaviour of red needle cast (Wake et al. 2018). To refine this model, a study was undertaken to monitor the progress of infection after foliage on three-year-old grafts of radiata pine was inoculated with *P. pluvialis* under assumed optimal conditions for the pathogen (Gómez-Gallego et al. 2019a). qPCR analysis and symptom severity indicated a small peak in detection after four days and a second larger peak at ca. 20 days, followed by a decline in detectable pathogen incidence.

In addition, field research has been conducted to determine the seasonal life cycles of both *Phytophthora* species. Between 2012 and 2014 spore traps consisting of freshly detached radiata pine fascicles floating on deionised or rainwater held in plastic containers were placed at fortnightly intervals beneath initially symptomatic radiata pine stands (Fraser et al. 2020). In the laboratory, sections of the needle baits were plated onto selective isolation media to establish the presence and identity of trapped phytophthoras. Inoculum was detected in most months throughout the year, although the pattern varied annually and with location. In some years, and depending on the site, inoculum of *P. pluvialis* was present from March (autumn) through to January (summer) and that of *P. kernoviae* from March through to November (spring). Peak abundance for both species was in late winter, approximately coincident with maximum disease expression nationally. Accordingly, probability of detection of inoculum was related to lower temperatures and periods of wet weather (Fraser et al. 2020). Similarly, preliminary small-scale studies with potted grafted cuttings have revealed successful intermittent infection at least between July and October (mid-winter and mid-spring; Hood et al. 2017). In a further study, relative abundance of *P. pluvialis* in Douglas-fir foliage at different locations was found to be positively correlated with mean winter relative humidity (Gómez-Gallego et al. 2019b).

This paper presents the results of a field trial conducted to gather confirmatory information on the seasonal life cycle of *Phytophthora pluvialis* and *P. kernoviae* on radiata pine. A succession of radiata pine exchange plants were deployed to field sites to determine when infection (as measured by visible symptoms and presence of pathogens) occurs and to examine how this relates to weather variables. To confirm species identity, two procedures, high-throughput qPCR and plating onto selective media, were used to detect the *Phytophthora* pathogens from a subset of needle samples taken from the study plants. Spore traps were included to enable a comparison with earlier work (Fraser et al. 2020).

Methods

Trial period

The trial was run in two contiguous phases, the first between late November 2017 and November 2018, and the second between November 2018 and early January 2020 (Fig. 1).

Sites

Four sites were established in mature radiata pine stands showing symptoms of red needle cast in two forests in the Central North Island approximately 50 km apart. Sites 1 ("Tar Hill"; Lat: -38.30251; Long: 175.95880) and 2 ("Kaki Road"; Lat: -38.36274; Long: 175.91888), 8 km apart, were located in Kinleith Forest. Sites 3 ("Goudies Road"; Lat: -38.43808; Long: 176.49657) and 4 ("Low Level Road"; Lat: -38.61988; Long: 176.49988), 21 km apart, were located in Kaingaroa Forest. Due to

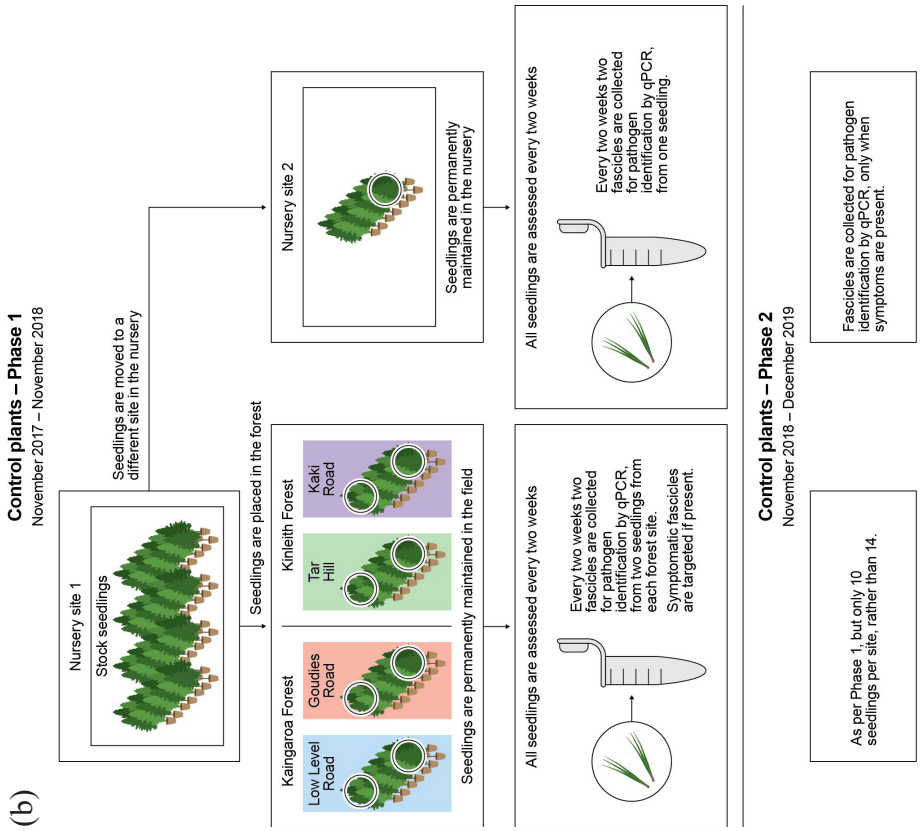

FIGURE 1: Design of the red needle cast infection timing trial (schematic). (a) Exchange plants. (b) Controls.

operational felling, Site 1 ("Tar Hill") was relocated 700 m to a new position for the second phase (Lat: -38.30497; Long: 175.96530) in a new stand also affected by red needle cast at that time.

Infection period

Plant material

Potted, open-pollinated, GF 19 (Vincent 1987; NZFFA 2005), radiata pine seedlings untreated with fungicides were exposed to natural inoculum at successive fortnightly intervals to detect when infection occurred. Plants were lifted from nursery beds, individually potted in 9 L plastic pots and held for a short period until stabilised prior to use. A different set of plants, each of one seed lot, was deployed during each phase of the study. Seedlings ranged 30–100 cm in height during the trial period.

Exchange plants

Sets of potted seedlings were transported to the field for two weeks before being replaced by new plants, the replaced set being returned to a different location in the nursery (Fig. 1a). Seven seedlings were exchanged at each site per fortnight. Seedlings returned to the nursery were assessed every two weeks for 12 (first phase) or 10 (second phase) weeks before being discarded (Fig. 1a). The number or percentage of needles on each plant with symptoms of red needle cast infection were scored on the following scale: 0, none; 1, 1-10 needles; 2, >10 needles but <50% of needles; 3, > 50% of needles.

Control plants

In addition, 14 seedlings (first phase) and 10 seedlings (second phase) were kept permanently at each of the four field sites as positive controls (Fig. 1b). Positive control seedlings were replaced by fresh plants if they became unhealthy due to prolonged shading from the stand canopy or infection by *Phytophthora*. Replacements began in May in the first phase and February in the second phase. Fourteen seedlings were held permanently in the nursery throughout the trial as negative controls, in an area separate from the exchange seedlings. All plants were exposed to natural rainfall and were watered as necessary from beneath in the field and from above in the nursery.

Control seedlings were assessed every two weeks throughout each study phase using the same procedure as for the exchange plants (assisted for the shaded positive field controls by torchlight illumination).

Pathogen identification

During the first phase of the trial, needle fascicles were sampled at each assessment and prepared for detection of *P. pluvialis* and *P. kernoviae* by automated high-throughput DNA extraction and species-specific qPCR targeting the *Ypt*1 gene region (Schena et al. 2006; McDougal et al. 2021) at Slipstream Automation, Palmerston North (O'Neill et al. 2018). Sampling was prioritised towards symptomatic needles on seedlings with such foliage. Two fascicles were sampled from each of two plants per seedling batch (i.e., 2 fascicles × 2 plants × 4 sites × 6 fortnightly exposure intervals = 48 two-fascicle samples every two weeks, once the trial was underway). Each fortnight, two fascicles were also collected from at least two field control seedlings at each of the four sites and at least one seedling from the nursery negative control set.

When symptoms were observed, isolations onto *Phytophthora*-selective media were attempted in addition to qPCR (which was undertaken whether symptoms were present or not). In these cases, of the two-fascicle sample per plant, one was used for qPCR and one for isolation. To isolate the pathogens, sections of needles 5 mm long were surface sterilised for 30 seconds in 70% ethanol, rinsed twice in sterile deionised water, blotted dry in clean paper towelling and plated onto 10% carrot agar (CA) amended with 0.2 g/L ampicillin, 0.05 g/L nystatin, 0.01 g/L rifampicin and 0.01 g/L pimaricin (Gómez-Gallego et al. 2019a). Sections were selected to include the margins of characteristic red needle cast lesions. Emerging colonies typical of *Phytophthora* were sub-cultured on CA (Dick et al. 2006) and identified based on macro- and micromorphological features.

During the second phase of the trial, needle samples were taken only when symptoms were observed, and these were analysed solely by qPCR. Disease severity was low in the second year and this procedure avoided possible confusion between young lesions produced by *Dothistroma septosporum* and those of red needle cast. Symptoms of RNC were only recorded as present when either *Phytophthora* species was detected by qPCR.

Spore traps

During the first phase of the study, spore traps were also set up and monitored at each site to allow comparisons with data from the exchange plant study and with the previous inoculum timing study of Fraser et al. (2020). These consisted of square plastic buckets of cross-sectional dimensions 25 × 25 cm, covered in a coarse, ca. 1 cm square, plastic coated wire grid to exclude litter, and holding about 5 L deionised water. Traps were placed on the ground at the study sites (5 traps per site) and were baited with freshly collected needle fascicles of radiata pine held in coarse mesh bags floating on the surface of the deionised water. Fascicles were taken at the same position from a GF 19 plant of a seed lot known from detached needle inoculation assays to be receptive to colonisation by *P. pluvialis*, avoiding new growth. These were held overnight at 4°C and transported wrapped in fresh dry paper towelling inside clean polythene bags within an insulated polystyrene container for placement in traps the following day. Baits and deionised water were changed fortnightly, and on return to the laboratory baits were again held overnight at 4°C prior to processing the next day. Bags were soaked in bleach, rinsed thoroughly with water and dried prior to reuse.

Baits were evaluated by isolation and morphological identification of resulting cultures, as described above. In addition, isolations were attempted each fortnight from fresh needles from the bait source plant as negative controls. Positive controls consisted of isolation attempts made from needles exposed each fortnight as baits to *Phytophthora* zoospores in the laboratory. Bait needles

were placed along with 5 mm diameter CA plugs from a standard *P. pluvialis* culture, and later (from early July 2018) also from a *P. kernoviae* culture, in sterile pond water to induce production of sporangia and release of zoospores. Ten needle bait sections were plated per trap and for each control at each fortnightly interval.

Weather variables

The National Institute of Water and Atmospheric Research (NIWA) provides daily meteorological estimates for points on a Virtual Climate Station Network (VCSN) spatially interpolated using actual data from real climate stations located around New Zealand (Tait et al. 2006; https://www.niwa.co.nz/climate/our-services/virtual-climate-stations). Data for the following variables were extracted from the virtual 5 km-grid weather station nearest to each site for the period from November, 2016: daily maximum air temperature (°C), daily minimum air temperature (°C), daily soil temperature (°C), rain accumulation over 24 hr (mm), relative humidity (RH) at 9.00 a.m. (%), solar radiation over 24 hr (MJ/m^2), mean wind speed over 24 hr at 10 m (m/sec.) and Penman's evapotranspiration index over 24 hr (kg/m^2; Penman 1948).

Data analysis

The analyses of infection were run as one data set from November 2017 to January 2020. Statistical analyses were conducted using R 3.6.2 (2019).

NIWA virtual weather station data were used to predict RNC infection, as expressed by the presence of symptoms, on the foliage of exchange seedlings during the study period. Plants were treated as infected if symptoms were recorded at least once during assessments after being returned from the field. Fortnightly lag variables were constructed so that the proportion of seedlings at each exchange period that developed RNC symptoms at each site could be compared with historical as well as concurrent weather (Table S1). Lag variables of time periods T1 to T26 represented weather from 1 to 26 fortnights prior to seedling exchanges. Because variables for predicting RNC at each exchange period at each site were correlated, gradient boosting machine learning (gbm) analyses were used to identify the most important weather variables using the R package gbm (Friedman 2002; Greenwell et al. 2020). Tree-based analyses such as gradient boosting models are suited to analysis of data with high collinearity among variables (Dormann et al. 2013). A Gaussian distribution with 100 trees was used to specify the gbm model. Calculation of goodness of fit statistics (RMSE, R^2) and diagnostics were undertaken.

Generalised mixed effects models, generalised least square (GLS) models and ordinary least square (OLS) models were fitted to the most important variables, identified for each model by gradient boosting analysis. GLS models included a serial correlation matrix to allow for the effects of temporal autocorrelation. An automated stepwise procedure was applied to choose the minimum adequate model, using AIC as a selection criterion. The most parsimonious model which adequately predicted the relationship between important weather variables and the proportion of RNC symptomatic seedlings was an ordinary least squares regression. Inclusion of variables identifying the season when RNC was measured, the site, or temporal autocorrelation, did not significantly improve the most parsimonious models. Adjusted R^2 values were calculated following Nakagawa et al. (2017).

To investigate seasonal variation in rates of symptom development, the time taken before RNC symptoms appeared, after seedlings were returned from the field, was plotted against time of year. An apparent difference between rates in winter and spring in the first phase of the study was analysed using a t-test.

Comparison of pathogen detection data from qPCR and isolation onto selective media was assessed with a McNemar's test of contingency table for *P. pluvialis* and *P. kernoviae* separately. A continuity correction was applied due to low numbers of positives.

Because positive detections from spore traps were low in number, this dataset is presented but was not analysed statistically.

Results

Seasonal pattern of symptom development and pathogen detection

Symptoms of RNC appeared on exchange seedlings during both phases of the trial (Fig. 2a). They occurred predominantly on plants exposed between April and September (mid-autumn to early-spring) in 2018 and between April and July (mid-autumn to mid-winter) in 2019 (Fig. 3). Fewer exchange seedlings developed symptoms during the second phase. Symptoms were also observed during the first phase on a plant exposed at Tar Hill between 19 December 2017 and 15 January 2018 (Fig. 3).

Phytophthora pluvialis was first detected on a symptomless seedling exposed at Low Level Road between 20 November and 5 December 2017 (Fig. 3). The earliest detection of infection by *P. kernoviae* was made on the seedling that showed symptoms after exposure between 19 December 2017 and 15 January 2018. However, the main period during the first phase in which the phytophthoras were detected on exchange plants was between April and September 2018 for *P. kernoviae*, and between April and August 2018 for *P. pluvialis*. During the second phase, *P. kernoviae* was detected between April and July, 2019, but *P. pluvialis* was only detected in one fortnight in July 2019 (Fig. 3).

Control seedlings

On field seedlings permanently exposed to available inoculum under conditions of perpetual shade (positive controls), disease symptoms differed somewhat from those on exchange seedlings, which were only shaded during the fortnight in which they were kept in the field (Fig. 2b, c). Nevertheless, these symptoms on control plants were observed during a similar period to that for exchange plants. During the first phase, symptoms on most control seedlings were recorded between June and November 2018 (early winter through to late spring), when the plants were replaced for the second phase of

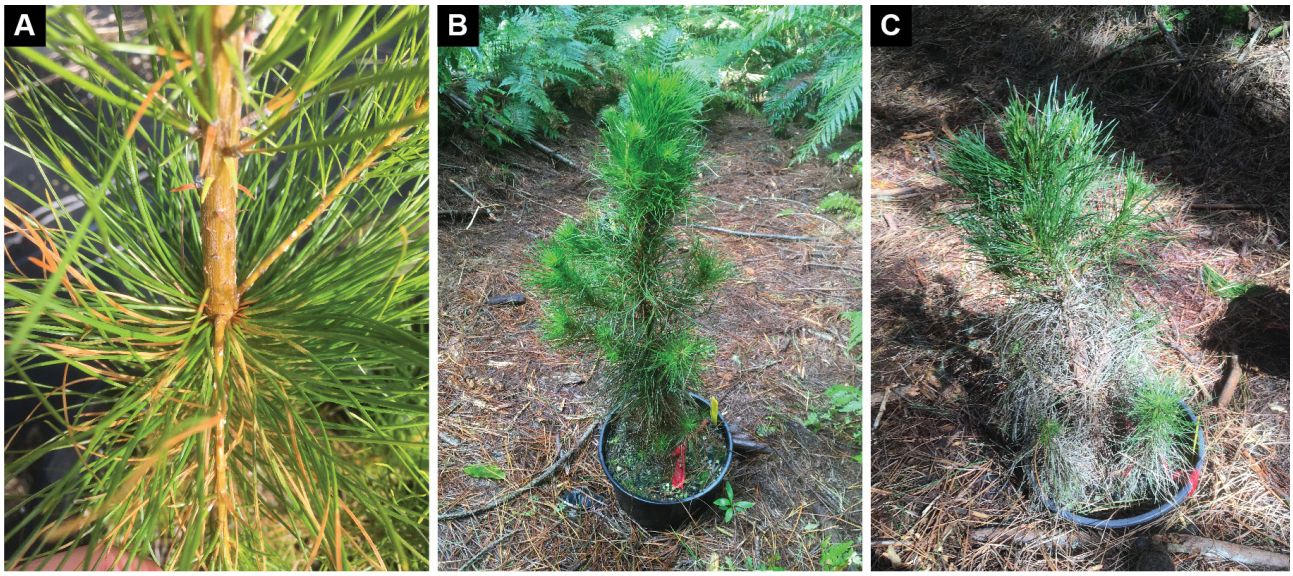

FIGURE 2: Symptoms of *Phytophthora* infection on foliage of radiata pine seedlings in the present study. (a). Typical symptomology on an exchange seedling after its return to an open section in the nursery. Affected portions of needles have transitioned from olive green to khaki-orange-red. (b, c). Atypical symptoms as seen on shaded field control seedlings maintained under the forest canopy. On such plants affected foliage often first turned dark green and then grey rather than transitioning to red as is more characteristic for the disease on canopy trees.

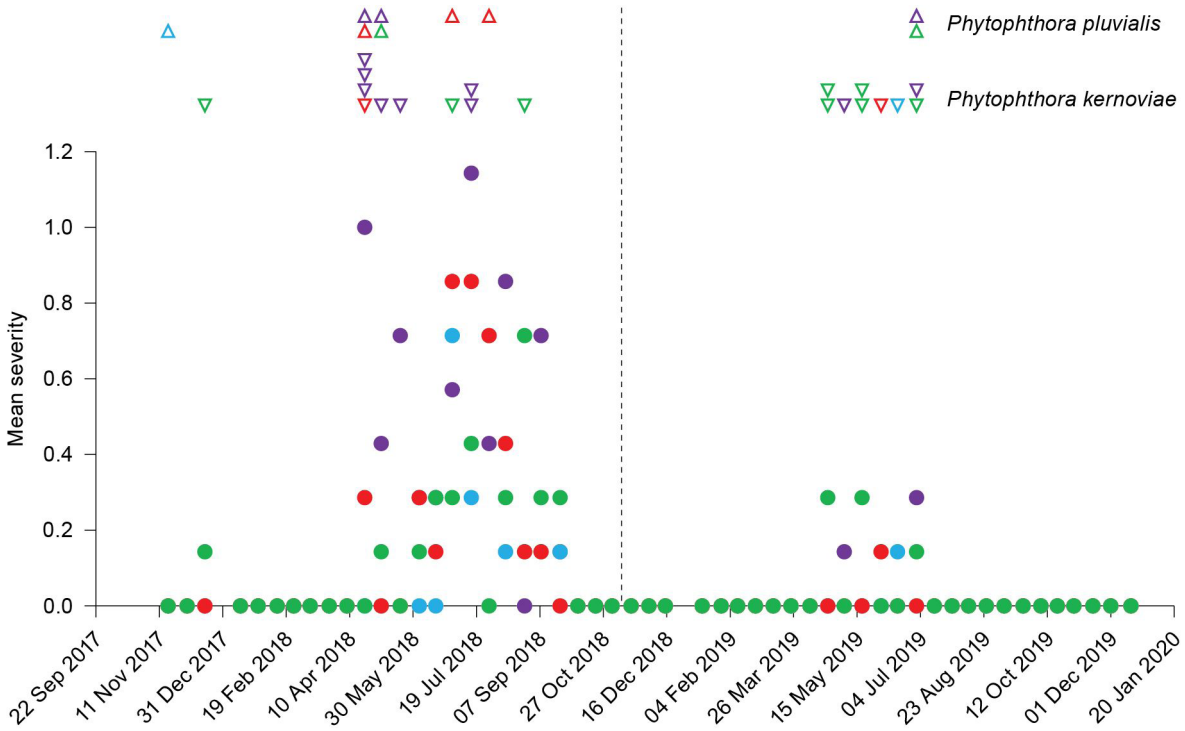

FIGURE 3: Severity of RNC symptoms by time of year on exchange seedlings. Each dot indicates the mean, for all exchange seedlings exposed at a specific site and fortnight, of the highest score per plant (full symptom expression) from the series of assessments made after returning from the field (note: not all zero value dots are visible where they coincide and are superimposed). Scale (needles with symptoms): 0, none; 1, 1-10 needles; 2, >10 needles but <50% of needles; 3, > 50% of needles. Also shown are positive detections of *P. pluvialis* or *P. kernoviae* in needle samples taken from exchange seedlings exposed at specific sites and fortnights using qPCR and/or isolation (each symbol represents detection on one plant; negative qPCR results are not shown, including those for 328 samples from seedlings exposed between 15 January 2018 and 23 April 2018). Sites: Kinleith Forest: green, Tar Hill; purple, Kaki Road. Kaingaroa Forest: red, Goudies Road; blue, Low Level Road. Each point indicates the starting date of its fortnightly period of exposure. The vertical dotted line separates seedlings of the first and second phases of the study.

the study (Fig. 4). During the second phase, symptoms were observed on the newly deployed plants between December 2018 and January 2019 (summer), with a lull preceding a fresh period with symptoms recorded from May 2019 to January 2020 (early winter to summer), comparable to that in the first year. On the permanently exposed control seedlings, *P. pluvialis* was detected by qPCR between July and November, and *P. kernoviae* between May and November, during the first phase (Fig. 4). During the second phase, *P. pluvialis* was detected between December 2018 and January 2019, and again between May 2019 and January 2020, while *P. kernoviae* was detected in January 2019 and then between July 2019 and January 2020 (Fig. 4).

No symptoms of RNC developed on negative control seedlings held permanently in the nursery. Likewise, neither species of *Phytophthora* was detected by qPCR on samples collected from negative control plants.

Observed relationship with meteorological variables

Symptom expression and pathogen detection on exchange seedlings were greatest in both forests between April and September (late autumn through to mid spring), when air and soil temperatures, solar radiation and evapotranspiration were at their lowest, and relative humidity was at its maximum (Figs. 3, 5a,b,d-g). Rainfall occurred intermittently but was still ample during the period when infection and pathogen detection occurred (Figs. 3, 5c).

Analysis of the relationship with meteorological variables

A gradient boosting model with predictor variables of site and fortnightly lags for evapotranspiration, maximum temperature, minimum temperature, rainfall, relative humidity, photosynthetically active solar radiation, soil temperature and wind speed identified four variables with importance scores over 5%. These were soil temperature from 13 to 15 fortnights before the exchange, and minimum temperature in the fortnight before exchange (Table S2). The full model explained 74% of variation in data (Table 1, RMSE = 0.170, R^2 value of 0.739; Fig. 6b). An OLS model containing the ten most important variables identified in the gradient boosting model accounted for 36% of variability in RNC scores (RMSE = 0.134, R^2 value of 0.357; Table 1;

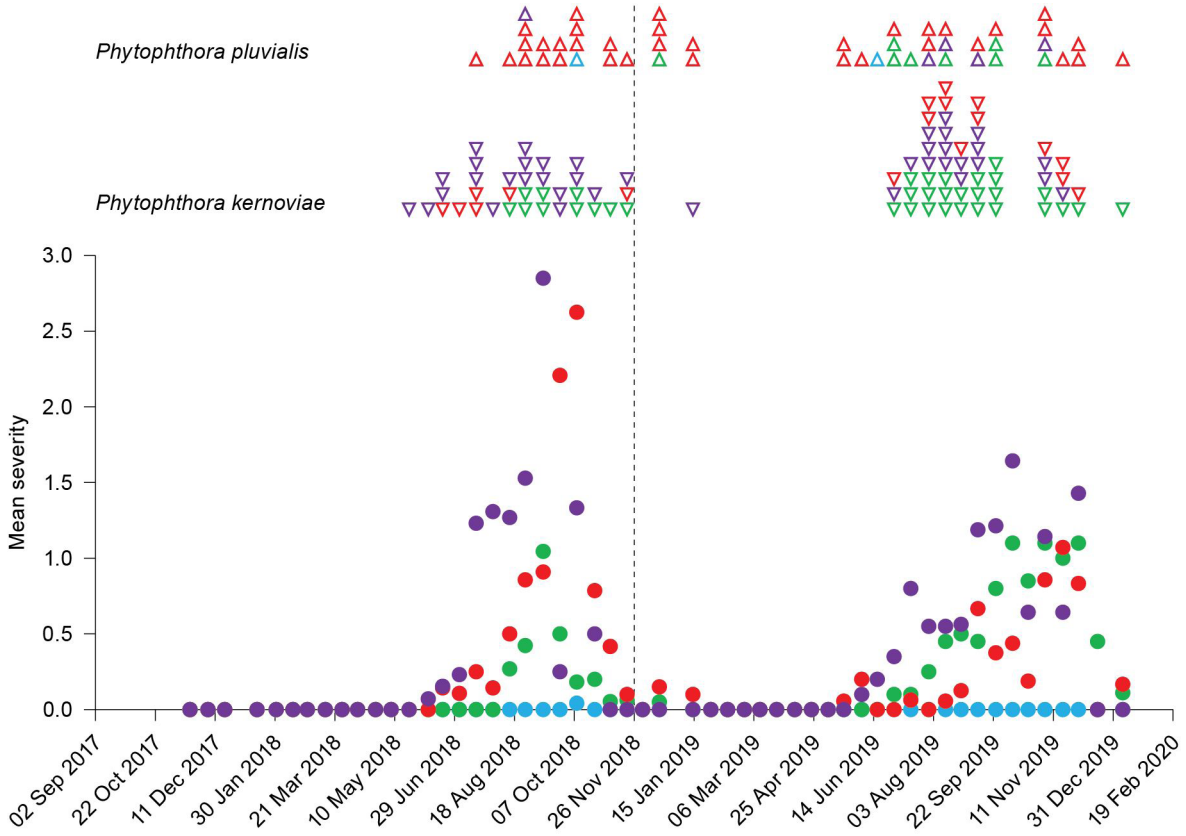

FIGURE 4: Severity of RNC infection by time of year on field control seedlings. Each dot indicates the mean score for all permanently placed plants at a specific site and date (up to 10 or 14 plants per site, depending on year and survival; note: not all zero value dots are visible where they coincide and are superimposed). Scale (needles with symptoms): 0, none; 1, 1-10 needles; 2, >10 needles but <50% of needles; 3, > 50% of needles. Also shown are positive detections of *P. pluvialis* or *P. kernoviae* in needle samples taken from control seedlings at specific sites and times using qPCR and/or isolation (each symbol represents detection on one plant; negative qPCR results are not shown, including those for 55 samples taken from 5 December 2017 to 7 May 2018). Sites: Kinleith Forest: green, Tar Hill; purple, Kaki Road. Kaingaroa Forest: red, Goudies Road; blue, Low Level Road. The vertical dotted line separates seedlings of the first and second phases of the study.

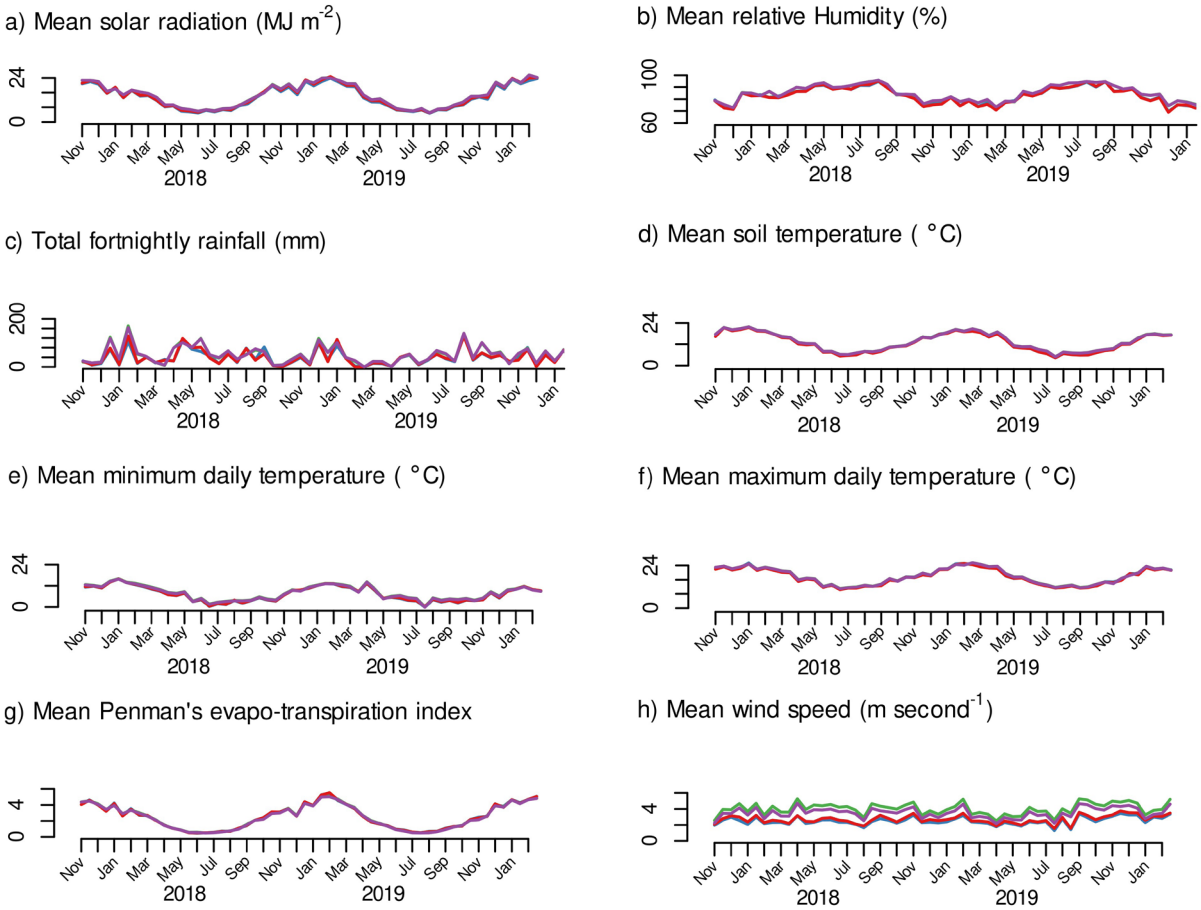

FIGURE 5: Seasonal weather patterns during the trial. Fortnightly means of data from the nearest NIWA virtual weather station to each site. Kinleith Forest: green lines, Tar Hill; purple lines, Kaki Road. Kaingaroa Forest: red lines, Goudies Road; blue lines, Low Level Road.

Fig. 6c). A stepwise procedure reduced the number of predictor variables included in the linear model to four, with little difference in the model fit (RMSE = 0.135, R^2 value of 0.335; Table 2). Soil temperatures 13 and 14 fortnights prior to the exposure period had positive relationships with the presence of symptoms. Maximum air temperature 14 fortnights prior and relative humidity 20 fortnights prior to exposure had negative relationships with the presence of symptoms. Caution should be applied to results from linear regression using correlated predictor variables, even of a reduced number.

TABLE 1: Root mean square error (RMSE) and R^2 statistics from models used to predict RNC symptoms. The gradient boosting model included 212 highly correlated predictor variables. The most important of these were used in linear regression models. Other methods were tried including Nagelkerkes R^2 and from packages including ModelMetrics. DescTools, fmsb.

Model	RMSE [a]	R^2 [b]	Nagelkerke
Gradient boosting	0.170	0.753	
Binomial General Linear Model (GLM)	4.886	0.331	0.465
OLS Linear model	0.134	0.357	
Stepwise OLS	0.135	0.350	

[a] $\sqrt{mean|(predicted - observed)^2}$

[b] correlation of (observed vs fitted)^2

Period between field exposure and symptom expression

During the first phase of the trial, time until symptoms appeared was significantly greater on seedlings exchanged before August (i.e., exposed in mid-winter; mean, 2.9 fortnights) than on those exposed later (i.e. exposed in early spring; mean, 1.3 fortnights; t = 5.584, P < 0.001; Fig. S1). After August, a greater number of plants were already symptomatic when returned from the field. No trends were apparent among the limited positive disease data obtained during the second phase (Fig. S1).

Seasonal pattern of detection of *Phytophthora* spp. in spore traps

Inoculum of *Phytophthora* was detected only infrequently in the spore traps during the trial (undertaken during the first phase, only), but when present it matched the seasonal timing for infection and RNC symptom

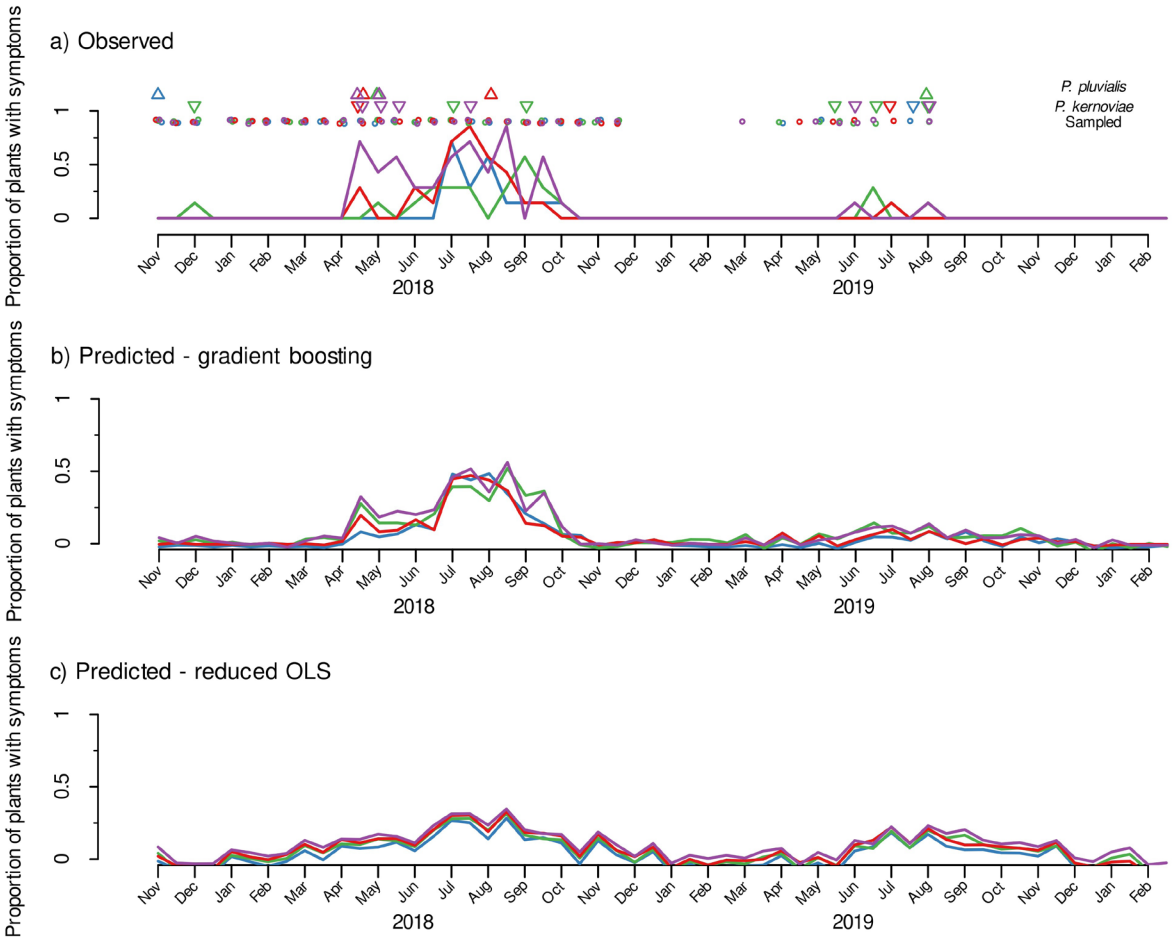

FIGURE 6: Seasonal development of RNC. Proportion of exchange seedlings with RNC symptoms: actual and predicted data from gradient boosting (gbm) and reduced OLS models fitted to weather data. Also shown are dates of needle sampling including those with qPCR detection of *P. pluvialis* and *P. kernoviae*. From four sites in two forests (Kinleith Forest: green line, Tar Hill; purple line, Kaki Road. Kaingaroa Forest: red line, Goudies Road; blue line, Low Level Road).

development on exchange seedlings (Fig. 7). Inoculum of *P. pluvialis* was identified during August (late winter; in Kaingaroa Forest) and *P. kernoviae* between June and August (throughout winter; in Kinleith Forest). Neither species was isolated from negative control needles. Of the 10 positive control needle sections plated each fortnight, *P. pluvialis* was obtained from a mean of 5.9 sections (range 0-10; n=24) and *P. kernoviae* from a mean of 3.4 sections (range 1-8; n=10).

Comparison of pathogen detection methods

There was greater percentage detection by automated high-throughput qPCR than isolation onto *Phytophthora*-selective media for both *Phytophthora* species from a subset of 64 samples from the first phase of the trial. *Phytophthora pluvialis* was detected from 7.8% of samples by qPCR compared to 3.1% of samples by isolation. *Phytophthora kernoviae* was detected from 9.4% of samples by qPCR compared to 7.8% of

TABLE 2: ANOVA table from the OLS linear model stepwise procedure. Regression coefficients are displayed for the four variables selected by the procedure. Lag variables are described from 1 to 26 fortnights prior to the exposure fortnight.

Parameter	df	Mean Sq	F value	P	Coefficient	SE Coeffcient
Site	1	0.126	6.703	0.01	0.021	0.008
Soil Temperature Week 14	1	1.325	70.751	0	0.044	0.009
Soil Temperature Week 13	1	0.11	5.864	0.016	0.019	0.006
Maximum Air Temperature Week 14	1	0.517	27.622	0	-0.057	0.01
Relative humidity Week 20	1	0.118	6.305	0.013	-0.005	0.002
Residuals	218	0.019				

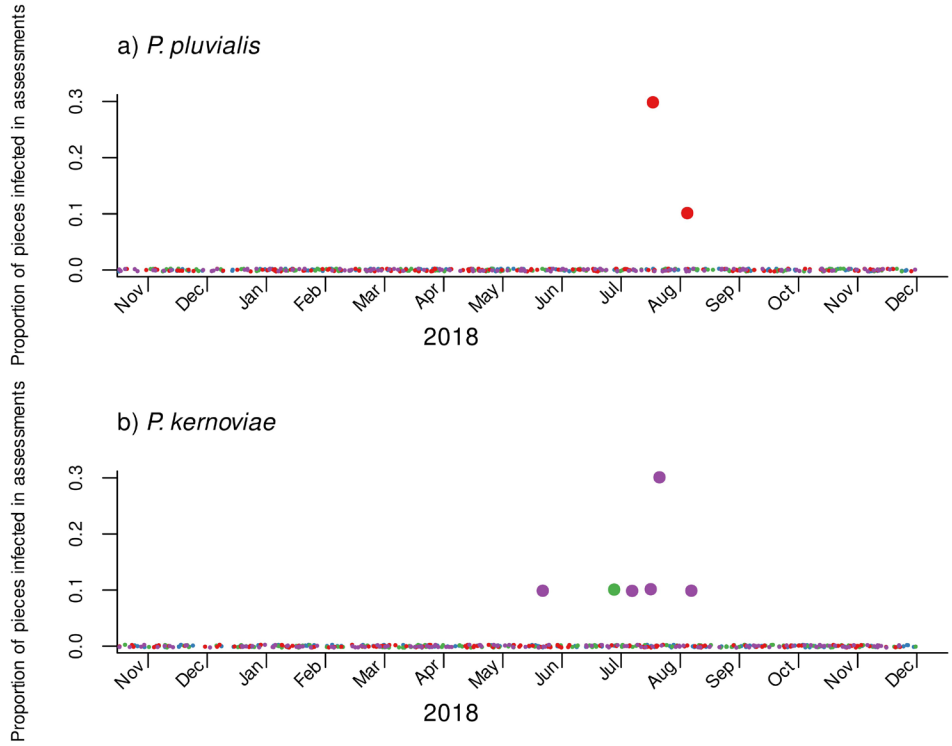

FIGURE 7: Seasonal pattern of detection of *Phytophthora* species in inoculum spore traps at four sites in two forests. Each dot indicates the proportion of 10 fragments from needles in one trap yielding (a) *Phytophthora pluvialis* or (b) *Phytophthora kernoviae* (five traps per site). Kinleith Forest: green dots, Tar Hill; purple dots, Kaki Road. Kaingaroa Forest: red dots, Goudies Road; blue dots, Low Level Road.

samples by isolation. However, these differences were not statistically significant (McNemar's test, $P > 0.05$). *Phytophthora pluvialis* was not detected by isolation from samples that were also negative by qPCR. However, *P. kernoviae* was isolated from two samples that were negative for the species as determined by qPCR. Only three of 35 positive detections from exchange seedlings, and three of 122 from field control seedlings, had no records of symptoms being present.

Discussion

The results from this trial demonstrated a seasonal pattern of RNC development that corroborates results from earlier studies, implying that most infection by *P. pluvialis* and *P. kernoviae* takes place between autumn and spring, tailing off into summer especially in years when RNC is more severe. During the first phase of the study, infection in the exchange plants, as determined by the qPCR analysis and symptom expression, occurred mainly between April (autumn) and September (early spring), with some in November and December 2017 (spring-early summer). No infection was detected between late January and mid-April 2018 on the many samples (328) that underwent qPCR during that period and no symptoms were recorded. Infection also occurred in late November or December 2018 on the newly placed second phase control plants, with some *possibly* extending through to January 2019. This pattern was clearly apparent even though both phases of the trial were conducted during a low disease period following two years of severe disease expression in each forest. It is likely that in years of greater severity some infection may occur both earlier and later than indicated in this study. The brief incidence of infection detected in exchange seedlings exposed during November-December 2017 and December 2017-January 2018 at the beginning of the first phase may have been the residual aftermath of the previous, more severe period of RNC. The qPCR and isolation results supported earlier work showing that the life cycles of *P. pluvialis* and *P. kernoviae* are similar, and as with some other phytophthoras they are apparently polycylic. This trial did not include a micromorphological aspect, but empty sporangia of *P. pluvialis* were observed part way through an initial pilot study on the surface of a needle from an exchange plant following earlier infection in the same season (Hood et al. 2017). This observation and the sustained detection of inoculum in previous spore trap work signify the repetitive production of infectious propagules during the infection period (Fraser et al. 2020). RNC thus progresses epidemically, especially in high disease severity years, as the season advances.

A key aim of the present trial was to investigate how the infection periods of *P. pluvialis* and *P. kernoviae* are affected by different weather variables. The results of the study concur with previous work showing that infection mostly takes place during the cooler, wetter winter months, when relative humidity is greater, temperatures, solar radiation and evapotranspiration are lower, at times of ample rainfall and foliage wetness (Fraser et al. 2020). It also appears that symptoms on

infected needles developed more rapidly later in the season, as winter transitioned into spring. The seasonal relationship between infection (measured as proportion of plants with visible symptoms) and weather was examined statistically. The best model accounted for 33% of the variation in symptom expression which was explained by four key weather variables prevailing in the six months before seedlings were exposed. However, it is unclear from these observations which variables are the actual drivers because of their covariation, e.g., between warmer summer temperatures and increased solar radiation (this particular relationship might be less likely with the plants in this study, however, as they were shaded beneath mature trees). The models did not identify a simple and clear association between any single weather variable and RNC. Because of this it will be necessary to conduct further experimental work. Follow up studies should focus on epidemic periods of the year, placing exchange plants directly under symptomatic canopy trees and utilising significantly shorter exposure periods (e.g., two days) to identify key variables for spore release, spread and infection. Further, the results of controlled environment inoculation studies will determine which climatic variables are primarily causative and, complementary to those of the present and previous research, thereby helping to clarify RNC epidemiology (Gómez-Gallego et al. 2019a; Fraser et al. 2020).

Direct evaluation by means of automated high-throughput qPCR was a more efficient technique than isolating phytophoras from needles, in agreement with Gómez-Gallego et al. (2019a,b) and Fraser et al. (2022). Only two samples yielding cultures of *P. kernoviae* tested negative for this species using qPCR, possibly due to the low level of disease during the trial period, with often only a single needle on one of the two sampled fascicles displaying symptoms. Both methods were better indicators of inoculum release and availability (since infection presupposes inoculum) than the spore trap procedure. It is puzzling why the spore traps gave only limited results, but this may also have been partly due to the low level of disease in the stands sampled and consequent reduced inoculum loading. Detached needle baiting was used successfully in the earlier study reported by Fraser et al. (2020). In that work spores were trapped over a period broadly comparable to that when infection occurred in this study. This suggests that absence of infection on exchange plants was due to a lack of inoculum, not because foliage was unreceptive to spores at this time, but this requires confirmation. It is still possible that spores may be released over a longer period than detected even in the spore trapping study of Fraser et al. (2020). It may be necessary to replace the present inoculum trapping method by a more sensitive procedure in future studies. Less inoculum during a low disease year may explain the reduced infection during the second phase of the exchange plant study, as determined by qPCR analysis supported by symptom expression. The very localized distribution of the disease may have also had an impact, with symptoms often not developing on canopy trees directly above the exchange seedlings, but on other canopy trees nearby. There is increasing evidence that most RNC inoculum remains local and disperses over only a short distance from its source (Hood et al. 2017).

The severity of a polycyclic epidemic is governed by the level of initial inoculum and the apparent rate of infection as the disease develops (Van der Plank 1963). For RNC we are still hampered by limited knowledge on both aspects, including the way the pathogens survive between outbreaks and the manner that spores are produced when the epidemic is initiated. *Phytophthora pluvialis* and *P. kernoviae* may survive in roots and/or soil (Gardner et al. 2015; Scott et al. 2019). It cannot be ruled out that in this study exchange seedlings positioned on the ground may have been exposed to some inoculum from this source as well as from the canopy. *Phytophthora pluvialis* does not appear to form resistant oospores readily in radiata pine needles (Hood et al. 2014; Scott et al. 2019), but it seems possible that a residue of viable infection persisting in tree crowns between disease events may serve as initial inoculum for a new disease episode when conditions are suitable. In this study, symptoms were present on some exchange and field control seedlings as late as January (regardless of when this foliage actually became infected), and Fraser et al. (2020) trapped inoculum in January in one trial year. Does a small level of infection continue on in plantation trees during the lull period between mid-summer and mid-autumn? It is noticeable that some disease appears to recur on the same groups of trees in successive years (I.A. Hood, unpublished data), although this observation may have other explanations. Control of the disease may eventually be achieved by both destruction of initial inoculum and reduction in the infection rate. Recent research indicates that one or two aerial spray applications of copper fungicide as early as November in the disease cycle are effective in reducing disease levels, as also are later applications (Fraser et al. 2022). The factors regulating disease outbreak years are still being determined, but it may ultimately be possible to advise when or when not to spray if weather conditions prior to the development of an epidemic govern the amount of initial inoculum. However, if weather variables during the development of an epidemic are more influential, or if aspects other than weather are also involved, this may not be achievable. Ultimately, a definitive outcome will also rely on further aerial fungicidal timing trials in a year when there is sufficient disease, in order to prescribe a recommended fungicide application programme.

Conclusions

Red needle cast proceeds epidemically as a seasonal polycyclic disease in stands of radiata pine in the Central North Island of New Zealand. During two mild disease years, infection of potted seedlings by the pathogens *P. pluvialis* and *P. kernoviae* occurred predominantly between mid-autumn and early spring. At this time of year, air and soil temperatures, solar radiation and evapotranspiration were at their lowest, relative humidity at its maximum, and rainfall, though intermittent was

generally plentiful. However, additional work is required to determine which of these weather variables have the greatest impact on sporulation, spore dispersal, infection and symptom development. Modelling predicted that air and soil temperatures approximately six months, and relative humidity approximately 10 months prior to infection were the most influential variables tested. Further studies to resolve the epidemiology of RNC in order to support disease control research are underway.

Competing interests

The authors declare that they have no competing interests.

Author contributions

The trial was coordinated by IAH and SF, who also participated in the field and laboratory. Technical work was conducted in the field by AWE, GT and LW and in the laboratory by JFG and CB. Statistical analyses were undertaken by SH. The paper was written by IAH, SF and SH, and the final draft accepted by all co-authors.

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Publicly available data access

R analysis code and data are available from the authors (S. Husheer) and on a GitHub repository: https://github.com/ScionResearch/ScionBiometricsPublic/tree/master/InfectionTrialRNC

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Supplementary Figure and Tables

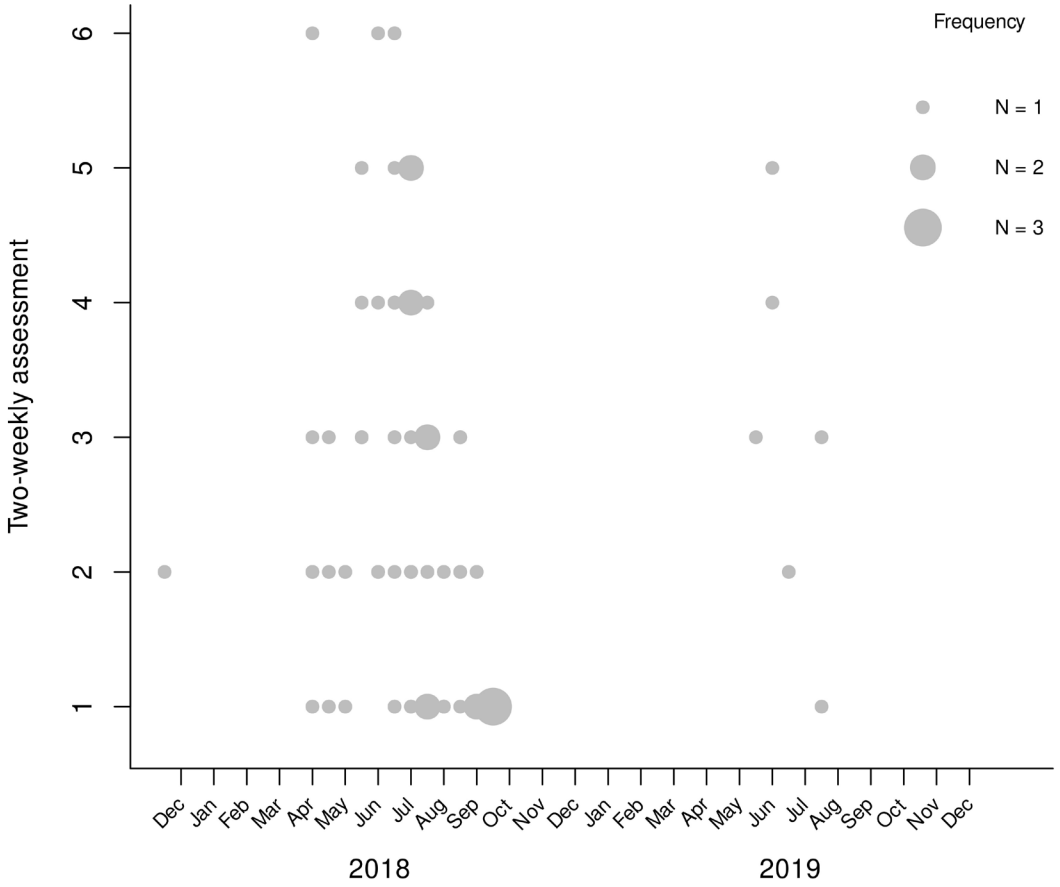

FIGURE S1: Dot plot showing development of RNC symptoms on exchange plants by time of year and period after exposure to inoculum. Horizontal axis: time of year when exposed. Vertical axis: period after return from field when symptoms first observed (in 2-week units; unit 1 indicates the first assessment made immediately on return, two weeks after initial placement in the field). Key: N=number of plants. During 2018, mean period before symptoms appeared prior to August (2.9 two-weekly intervals) was significantly longer than that after August (1.3 two-weekly intervals; $t = 5.584$, $P < 0.001$).

TABLE S1: Variables used in different models listed systematically. Final selection shows which variables were including in general and linear models. The general linear model is logistic. Variables identified as suitable for removal using the findCorrelation function from the Caret package. Note: "temp." is daily soil temperature, while "minTemp." and "maxTemp." refer to air temperature.

No.	variable	full.name	lag.char	var.char	FinalSelection	Correlated
1	evapoTrans	Mean Penman evapotranspiration (kg m-2)	Nil			Identified as highly correlated
2	evapoTrans.1	Mean fortnightly evapotranspiration - lag 1	1	evapoTrans		Identified as highly correlated
3	evapoTrans.10	Mean fortnightly evapotranspiration - lag 10	10	evapoTrans		Identified as highly correlated
4	evapoTrans.11	Mean fortnightly evapotranspiration - lag 11	11	evapoTrans		Identified as highly correlated
5	evapoTrans.12	Mean fortnightly evapotranspiration - lag 12	12	evapoTrans		Identified as highly correlated
6	evapoTrans.13	Mean fortnightly evapotranspiration - lag 13	13	evapoTrans		Identified as highly correlated
7	evapoTrans.14	Mean fortnightly evapotranspiration - lag 14	14	evapoTrans		Identified as highly correlated
8	evapoTrans.15	Mean fortnightly evapotranspiration - lag 15	15	evapoTrans		Identified as highly correlated
9	evapoTrans.16	Mean fortnightly evapotranspiration - lag 16	16	evapoTrans		Identified as highly correlated
10	evapoTrans.17	Mean fortnightly evapotranspiration - lag 17	17	evapoTrans		Identified as highly correlated
11	evapoTrans.18	Mean fortnightly evapotranspiration - lag 18	18	evapoTrans		Identified as highly correlated
12	evapoTrans.19	Mean fortnightly evapotranspiration - lag 19	19	evapoTrans		Identified as highly correlated
13	evapoTrans.2	Mean fortnightly evapotranspiration - lag 2	2	evapoTrans		Identified as highly correlated
14	evapoTrans.20	Mean fortnightly evapotranspiration - lag 20	20	evapoTrans		Identified as highly correlated
15	evapoTrans.21	Mean fortnightly evapotranspiration - lag 21	21	evapoTrans		Identified as highly correlated
16	evapoTrans.22	Mean fortnightly evapotranspiration - lag 22	22	evapoTrans		Identified as highly correlated
17	evapoTrans.23	Mean fortnightly evapotranspiration - lag 23	23	evapoTrans		Identified as highly correlated
18	evapoTrans.24	Mean fortnightly evapotranspiration - lag 24	24	evapoTrans		Identified as highly correlated
19	evapoTrans.25	Mean fortnightly evapotranspiration - lag 25	25	evapoTrans		Identified as highly correlated
20	evapoTrans.26	Mean fortnightly evapotranspiration - lag 26	26	evapoTrans		Identified as highly correlated
21	evapoTrans.3	Mean fortnightly evapotranspiration - lag 3	3	evapoTrans		Identified as highly correlated
22	evapoTrans.4	Mean fortnightly evapotranspiration - lag 4	4	evapoTrans		Identified as highly correlated
23	evapoTrans.5	Mean fortnightly evapotranspiration - lag 5	5	evapoTrans		Identified as highly correlated
24	evapoTrans.6	Mean fortnightly evapotranspiration - lag 6	6	evapoTrans		Identified as highly correlated
25	evapoTrans.7	Mean fortnightly evapotranspiration - lag 7	7	evapoTrans		Identified as highly correlated
26	evapoTrans.8	Mean fortnightly evapotranspiration - lag 8	8	evapoTrans		Identified as highly correlated
27	evapoTrans.9	Mean fortnightly evapotranspiration - lag 9	9	evapoTrans		Identified as highly correlated
28	maxTemp	Mean maximum daily air temperature C	Nil			Identified as highly correlated
29	maxTemp.1	Mean fortnightly daily maximum air temperature - lag 1	1	maxTemp	Included in linear model	Identified as highly correlated
30	maxTemp.10	Mean fortnightly daily maximum air temperature - lag 10	10	maxTemp		Identified as highly correlated
31	maxTemp.11	Mean fortnightly daily maximum air temperature - lag 11	11	maxTemp		Identified as highly correlated
32	maxTemp.12	Mean fortnightly daily maximum air temperature - lag 12	12	maxTemp		Identified as highly correlated
33	maxTemp.13	Mean fortnightly daily maximum air temperature - lag 13	13	maxTemp		Identified as highly correlated
34	maxTemp.14	Mean fortnightly daily maximum air temperature - lag 14	14	maxTemp	Selected by stepwise procedure for OLS	Identified as highly correlated
35	maxTemp.15	Mean fortnightly daily maximum air temperature - lag 15	15	maxTemp		Identified as highly correlated
36	maxTemp.16	Mean fortnightly daily maximum air temperature - lag 16	16	maxTemp		Identified as highly correlated
37	maxTemp.17	Mean fortnightly daily maximum air temperature - lag 17	17	maxTemp		Identified as highly correlated
38	maxTemp.18	Mean fortnightly daily maximum air temperature - lag 18	18	maxTemp		Identified as highly correlated

39	maxTemp.19	Mean fortnightly daily maximum air temperature - lag 19	19maxTemp		Identified as highly correlated
40	maxTemp.2	Mean fortnightly daily maximum air temperature - lag 2	2maxTemp		Identified as highly correlated
41	maxTemp.20	Mean fortnightly daily maximum air temperature - lag 20	20maxTemp		Identified as highly correlated
42	maxTemp.21	Mean fortnightly daily maximum air temperature - lag 21	21maxTemp		Identified as highly correlated
43	maxTemp.22	Mean fortnightly daily maximum air temperature - lag 22	22maxTemp		Identified as highly correlated
44	maxTemp.23	Mean fortnightly daily maximum air temperature - lag 23	23maxTemp		Identified as highly correlated
45	maxTemp.24	Mean fortnightly daily maximum air temperature - lag 24	24maxTemp		Identified as highly correlated
46	maxTemp.25	Mean fortnightly daily maximum air temperature - lag 25	25maxTemp		Identified as highly correlated
47	maxTemp.26	Mean fortnightly daily maximum air temperature - lag 26	26maxTemp		Identified as highly correlated
48	maxTemp.3	Mean fortnightly daily maximum air temperature - lag 3	3maxTemp		Identified as highly correlated
49	maxTemp.4	Mean fortnightly daily maximum air temperature - lag 4	4maxTemp		Identified as highly correlated
50	maxTemp.5	Mean fortnightly daily maximum air temperature - lag 5	5maxTemp		Identified as highly correlated
51	maxTemp.6	Mean fortnightly daily maximum air temperature - lag 6	6maxTemp		Identified as highly correlated
52	maxTemp.7	Mean fortnightly daily maximum air temperature - lag 7	7maxTemp		Identified as highly correlated
53	maxTemp.8	Mean fortnightly daily maximum air temperature - lag 8	8maxTemp		Identified as highly correlated
54	maxTemp.9	Mean fortnightly daily maximum air temperature - lag 9	9maxTemp		Identified as highly correlated
55	minTemp	Mean minimium daily air temperature C	Nil	Included in linear model	
56	minTemp.1	Mean fortnightly daily minimum air temperature - lag 1	1minTemp	Included in linear model	
57	minTemp.10	Mean fortnightly daily minimum air temperature - lag 10	10minTemp		
58	minTemp.11	Mean fortnightly daily minimum air temperature - lag 11	11minTemp		
59	minTemp.12	Mean fortnightly daily minimum air temperature - lag 12	12minTemp		
60	minTemp.13	Mean fortnightly daily minimum air temperature - lag 13	13minTemp		
61	minTemp.14	Mean fortnightly daily minimum air temperature - lag 14	14minTemp		
62	minTemp.15	Mean fortnightly daily minimum air temperature - lag 15	15minTemp		
63	minTemp.16	Mean fortnightly daily minimum air temperature - lag 16	16minTemp		
64	minTemp.17	Mean fortnightly daily minimum air temperature - lag 17	17minTemp		
65	minTemp.18	Mean fortnightly daily minimum air temperature - lag 18	18minTemp		
66	minTemp.19	Mean fortnightly daily minimum air temperature - lag 19	19minTemp		
67	minTemp.2	Mean fortnightly daily minimum air temperature - lag 2	2minTemp		
68	minTemp.20	Mean fortnightly daily minimum air temperature - lag 20	20minTemp		
69	minTemp.21	Mean fortnightly daily minimum air temperature - lag 21	21minTemp		
70	minTemp.22	Mean fortnightly daily minimum air temperature - lag 22	22minTemp		
71	minTemp.23	Mean fortnightly daily minimum air temperature - lag 23	23minTemp		
72	minTemp.24	Mean fortnightly daily minimum air temperature - lag 24	24minTemp		
73	minTemp.25	Mean fortnightly daily minimum air temperature - lag 25	25minTemp		
74	minTemp.26	Mean fortnightly daily minimum air temperature - lag 26	26minTemp		
75	minTemp.3	Mean fortnightly daily minimum air temperature - lag 3	3minTemp		
76	minTemp.4	Mean fortnightly daily minimum air temperature - lag 4	4minTemp	Included in linear model	
77	minTemp.5	Mean fortnightly daily minimum air temperature - lag 5	5minTemp		
78	minTemp.6	Mean fortnightly daily minimum air temperature - lag 6	6minTemp		
79	minTemp.7	Mean fortnightly daily minimum air temperature - lag 7	7minTemp		
80	minTemp.8	Mean fortnightly daily minimum air temperature - lag 8	8minTemp		

81	minTemp.9	Mean fortnightly daily minimum air temperature - lag 9		9minTemp	
82	rain	Fortnightly total rain fall (mm)	Nil		
83	rain.1	Total fortnightly rainfall - lag 1		1rain	
84	rain.10	Total fortnightly rainfall - lag 10		10rain	
85	rain.11	Total fortnightly rainfall - lag 11		11rain	
86	rain.12	Total fortnightly rainfall - lag 12		12rain	
87	rain.13	Total fortnightly rainfall - lag 13		13rain	
88	rain.14	Total fortnightly rainfall - lag 14		14rain	
89	rain.15	Total fortnightly rainfall - lag 15		15rain	
90	rain.16	Total fortnightly rainfall - lag 16		16rain	
91	rain.17	Total fortnightly rainfall - lag 17		17rain	
92	rain.18	Total fortnightly rainfall - lag 18		18rain	
93	rain.19	Total fortnightly rainfall - lag 19		19rain	
94	rain.2	Total fortnightly rainfall - lag 2		2rain	
95	rain.20	Total fortnightly rainfall - lag 20		20rain	
96	rain.21	Total fortnightly rainfall - lag 21		21rain	
97	rain.22	Total fortnightly rainfall - lag 22		22rain	
98	rain.23	Total fortnightly rainfall - lag 23		23rain	
99	rain.24	Total fortnightly rainfall - lag 24		24rain	
100	rain.25	Total fortnightly rainfall - lag 25		25rain	
101	rain.26	Total fortnightly rainfall - lag 26		26rain	
102	rain.3	Total fortnightly rainfall - lag 3		3rain	
103	rain.4	Total fortnightly rainfall - lag 4		4rain	
104	rain.5	Total fortnightly rainfall - lag 5		5rain	
105	rain.6	Total fortnightly rainfall - lag 6		6rain	
106	rain.7	Total fortnightly rainfall - lag 7		7rain	
107	rain.8	Total fortnightly rainfall - lag 8		8rain	
108	rain.9	Total fortnightly rainfall - lag 9		9rain	
109	rh.1	Mean fortnightly relative humidity - lag 1		1rh	
110	rh.10	Mean fortnightly relative humidity - lag 10		10rh	
111	rh.11	Mean fortnightly relative humidity - lag 11		11rh	
112	rh.12	Mean fortnightly relative humidity - lag 12		12rh	
113	rh.13	Mean fortnightly relative humidity - lag 13		13rh	
114	rh.14	Mean fortnightly relative humidity - lag 14		14rh	
115	rh.15	Mean fortnightly relative humidity - lag 15		15rh	
116	rh.16	Mean fortnightly relative humidity - lag 16		16rh	
117	rh.17	Mean fortnightly relative humidity - lag 17		17rh	
118	rh.18	Mean fortnightly relative humidity - lag 18		18rh	
119	rh.19	Mean fortnightly relative humidity - lag 19		19rh	
120	rh.2	Mean fortnightly relative humidity - lag 2		2rh	
121	rh.20	Mean fortnightly relative humidity - lag 20		20rh	Selected by stepwise procedure for OLS
122	rh.21	Mean fortnightly relative humidity - lag 21		21rh	

123	rh.22	Mean fortnightly relative humidity - lag 22		22rh		
124	rh.23	Mean fortnightly relative humidity - lag 23		23rh		
125	rh.24	Mean fortnightly relative humidity - lag 24		24rh		
126	rh.25	Mean fortnightly relative humidity - lag 25		25rh		
127	rh.26	Mean fortnightly relative humidity - lag 26		26rh		
128	rh.3	Mean fortnightly relative humidity - lag 3		3rh		
129	rh.4	Mean fortnightly relative humidity - lag 4		4rh		
130	rh.5	Mean fortnightly relative humidity - lag 5		5rh		
131	rh.6	Mean fortnightly relative humidity - lag 6		6rh		
132	rh.7	Mean fortnightly relative humidity - lag 7		7rh		
133	rh.8	Mean fortnightly relative humidity - lag 8		8rh		
134	rh.9	Mean fortnightly relative humidity - lag 9		9rh		
135	site	Site	Nil		Selected by stepwise procedure for OLS	
136	solar.1	Mean fortnightly photosynthetically active radiation - lag 1		1solar		Identified as highly correlated
137	solar.10	Mean fortnightly photosynthetically active radiation - lag 10		10solar		Identified as highly correlated
138	solar.11	Mean fortnightly photosynthetically active radiation - lag 11		11solar		Identified as highly correlated
139	solar.12	Mean fortnightly photosynthetically active radiation - lag 12		12solar		Identified as highly correlated
140	solar.13	Mean fortnightly photosynthetically active radiation - lag 13		13solar		Identified as highly correlated
141	solar.14	Mean fortnightly photosynthetically active radiation - lag 14		14solar		Identified as highly correlated
142	solar.15	Mean fortnightly photosynthetically active radiation - lag 15		15solar		Identified as highly correlated
143	solar.16	Mean fortnightly photosynthetically active radiation - lag 16		16solar		Identified as highly correlated
144	solar.17	Mean fortnightly photosynthetically active radiation - lag 17		17solar		Identified as highly correlated
145	solar.18	Mean fortnightly photosynthetically active radiation - lag 18		18solar		Identified as highly correlated
146	solar.19	Mean fortnightly photosynthetically active radiation - lag 19		19solar		Identified as highly correlated
147	solar.2	Mean fortnightly photosynthetically active radiation - lag 2		2solar		Identified as highly correlated
148	solar.20	Mean fortnightly photosynthetically active radiation - lag 20		20solar		Identified as highly correlated
149	solar.21	Mean fortnightly photosynthetically active radiation - lag 21		21solar		Identified as highly correlated
150	solar.22	Mean fortnightly photosynthetically active radiation - lag 22		22solar		Identified as highly correlated
151	solar.23	Mean fortnightly photosynthetically active radiation - lag 23		23solar		Identified as highly correlated
152	solar.24	Mean fortnightly photosynthetically active radiation - lag 24		24solar		Identified as highly correlated
153	solar.25	Mean fortnightly photosynthetically active radiation - lag 25		25solar		Identified as highly correlated
154	solar.26	Mean fortnightly photosynthetically active radiation - lag 26		26solar		Identified as highly correlated
155	solar.3	Mean fortnightly photosynthetically active radiation - lag 3		3solar		Identified as highly correlated
156	solar.4	Mean fortnightly photosynthetically active radiation - lag 4		4solar		Identified as highly correlated
157	solar.5	Mean fortnightly photosynthetically active radiation - lag 5		5solar		Identified as highly correlated
158	solar.6	Mean fortnightly photosynthetically active radiation - lag 6		6solar		Identified as highly correlated
159	solar.7	Mean fortnightly photosynthetically active radiation - lag 7		7solar		Identified as highly correlated
160	solar.8	Mean fortnightly photosynthetically active radiation - lag 8		8solar		Identified as highly correlated
161	solar.9	Mean fortnightly photosynthetically active radiation - lag 9		9solar		Identified as highly correlated
162	temp	Mean soil temperature C	Nil			Identified as highly correlated
163	temp.1	Mean fortnightly soil temperature - lag 1		1temp		Identified as highly correlated
164	temp.10	Mean fortnightly soil temperature - lag 10		10temp		Identified as highly correlated

165	temp.11	Mean fortnightly soil temperature - lag 11	11temp		Identified as highly correlated
166	temp.12	Mean fortnightly soil temperature - lag 12	12temp		Identified as highly correlated
167	temp.13	Mean fortnightly soil temperature - lag 13	13temp	Selected by stepwise procedure for OLS	Identified as highly correlated
168	temp.14	Mean fortnightly soil temperature - lag 14	14temp	Selected by stepwise procedure for OLS	Identified as highly correlated
169	temp.15	Mean fortnightly soil temperature - lag 15	15temp	Included in linear model	Identified as highly correlated
170	temp.16	Mean fortnightly soil temperature - lag 16	16temp		Identified as highly correlated
171	temp.17	Mean fortnightly soil temperature - lag 17	17temp		Identified as highly correlated
172	temp.18	Mean fortnightly soil temperature - lag 18	18temp		Identified as highly correlated
173	temp.19	Mean fortnightly soil temperature - lag 19	19temp		Identified as highly correlated
174	temp.2	Mean fortnightly soil temperature - lag 2	2temp		Identified as highly correlated
175	temp.20	Mean fortnightly soil temperature - lag 20	20temp		Identified as highly correlated
176	temp.21	Mean fortnightly soil temperature - lag 21	21temp		Identified as highly correlated
177	temp.22	Mean fortnightly soil temperature - lag 22	22temp		Identified as highly correlated
178	temp.23	Mean fortnightly soil temperature - lag 23	23temp		Identified as highly correlated
179	temp.24	Mean fortnightly soil temperature - lag 24	24temp		Identified as highly correlated
180	temp.25	Mean fortnightly soil temperature - lag 25	25temp		Identified as highly correlated
181	temp.26	Mean fortnightly soil temperature - lag 26	26temp		Identified as highly correlated
182	temp.3	Mean fortnightly soil temperature - lag 3	3temp		Identified as highly correlated
183	temp.4	Mean fortnightly soil temperature - lag 4	4temp		Identified as highly correlated
184	temp.5	Mean fortnightly soil temperature - lag 5	5temp		Identified as highly correlated
185	temp.6	Mean fortnightly soil temperature - lag 6	6temp		Identified as highly correlated
186	temp.7	Mean fortnightly soil temperature - lag 7	7temp		Identified as highly correlated
187	temp.8	Mean fortnightly soil temperature - lag 8	8temp		Identified as highly correlated
188	temp.9	Mean fortnightly soil temperature - lag 9	9temp		Identified as highly correlated
189	wind	Mean wind speed (m s-1)	Nil		
190	wind.1	Mean fortnightly wind - lag 1	1wind		
191	wind.10	Mean fortnightly wind - lag 10	10wind		
192	wind.11	Mean fortnightly wind - lag 11	11wind		
193	wind.12	Mean fortnightly wind - lag 12	12wind		
194	wind.13	Mean fortnightly wind - lag 13	13wind		
195	wind.14	Mean fortnightly wind - lag 14	14wind		
196	wind.15	Mean fortnightly wind - lag 15	15wind		
197	wind.16	Mean fortnightly wind - lag 16	16wind		
198	wind.17	Mean fortnightly wind - lag 17	17wind		
199	wind.18	Mean fortnightly wind - lag 18	18wind		
200	wind.19	Mean fortnightly wind - lag 19	19wind		
201	wind.2	Mean fortnightly wind - lag 2	2wind		
202	wind.20	Mean fortnightly wind - lag 20	20wind		
203	wind.21	Mean fortnightly wind - lag 21	21wind		
204	wind.22	Mean fortnightly wind - lag 22	22wind		
205	wind.23	Mean fortnightly wind - lag 23	23wind		
206	wind.3	Mean fortnightly wind - lag 3	3wind		

207	wind.4	Mean fortnightly wind - lag 4	4wind
208	wind.5	Mean fortnightly wind - lag 5	5wind
209	wind.6	Mean fortnightly wind - lag 6	6wind
210	wind.7	Mean fortnightly wind - lag 7	7wind
211	wind.8	Mean fortnightly wind - lag 8	8wind
212	wind.9	Mean fortnightly wind - lag 9	9wind

TABLE S2: Importance values measured (>1) calculated by residual sum of squares averaged over all trees of a gradient boosting model (gbm). All variables included in the gradient boosting model are listed in Table S1. Note: "temp." is daily soil temperature, while "minTemp." and "maxTemp." refer to air temperature.

variable	gbm.influence
temp.15	19.6820476
temp.14	10.2376401
minTemp.1	9.76337065
temp.13	5.35050889
minTemp	4.89797575
maxTemp.14	4.06793042
Rh	3.74165727
minTemp.4	2.8242143
rh.20	2.08245243
maxTemp.1	2.07143618
wind.18	1.94416014
rh.1	1.7698712
temp.17	1.73657758
rain.11	1.50218216
temp.1	1.4983119
evapoTrans	1.22943857
maxTemp.25	1.20768374
solar.18	1.20758296
temp.3	1.02308348
maxTemp.15	1.02027098
temp.2	1.01524595