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Infection period of red needle cast on *Pinus radiata*: fourth phase (2015-17)

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Cover photo

Inoculum trap and Infection indicator plant beneath a 13-year-old *Pinus radiata* stand at the time of trial establishment at Wharerata Forest. The floating-pine-needle-baited trap reveals when spores and/or sporangia of *Phytophthora pluvialis* are around and available. The plant determines when spores and/or sporangia cause infection under environmental conditions recorded by an adjacent weather station (not shown; see Fig. 3). Plants were topped in order to facilitate transport. Photo, Rod Brownlie, 11 August, 2015, Helen Track (Site 1).

Infection period of red needle cast on *Pinus radiata*: fourth phase (2015-17)

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March, 2017

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EXECUTIVE SUMMARY

1.1 The Problem.

Although the *Pinus radiata* foliage disease, red needle cast, has been present in New Zealand for at least nine years, there is still much to be learnt about its development and the life cycle of the causal pathogen, *Phytophthora pluvialis*. This information is fundamental to knowledge of when and how often to apply chemical control treatments. Studies to determine the infection period have been hampered by the sporadic nature of the disease, outbreaks of which occur only intermittently in different regions.

1.2 This Project

In order to improve the chances of success, a further study, the latest in a series, was set up at Wharerata Forest, a location where red needle cast has occurred more often and with greater severity than in some other parts of New Zealand. The trial was run over two periods in the latter part of 2015 and again during 2016. Potted grafted cuttings of two clones susceptible to the disease were placed at fortnightly intervals at two sites for periods of two weeks before being returned to the Scion nursery at Rotorua. This was to determine at which times of the year their foliage became infected. Floating pine needle baits in water-filled spore traps were similarly exchanged every two weeks in order to reveal indirectly when infective inoculum was available. To monitor stand disease, the source of the inoculum, tagged trees at each site were scored regularly for crown symptom severity. A solar powered recording meteorological station was placed at each site to enable comparison of results with local weather variables. In the second season an additional “subsite” was established during May, several hundred metres from each initial site, adjacent (within ca. 20 m) to groups of stand trees that were developing red needle cast crown symptoms.

1.3 Key Results

No stand disease, inoculum or infection on exchange plants were found during the first year of the study. In the second year disease developed at subsites but not at the original sites. During this period infection by *P. pluvialis* occurred on some exchange plants between the months of July and October, at the subsites close to diseased trees. This result does not preclude the possibility that infection may have been detected outside this period had a greater number of exchanged plants been used. Fertile sporangia of *P. pluvialis* were produced on infected needles. Inoculum was likewise detected at the subsites, only, during October. Infection occurred mostly during fortnights with higher rainfall (150mm or with ≥ 6 rain days) over the cooler period of the year when average minimum temperatures were less than 7 °C.

1.4 Final Conclusions and Implications of Results

These results, together with earlier spore trapping work (Nari Williams) support the view that in high severity years red needle cast develops as a polycyclic epidemic with initial infections taking place in early autumn. Repeated cycles of infection occur as the disease gradually intensifies through late winter and early spring eventually declining during summer as diseased needles are shed and new foliage matures, resulting in green trees with thin crowns. If this scenario is correct, it may be possible to manage the disease with an application of an effective, long-lasting fungicide that destroys initial inoculum early in autumn, and possibly a second some months later to maintain a low rate of infection.

1.5 Further Work

The above concept is speculative, based on minimal data, which at this stage are only indicative. A number of aspects require verification before precise recommendations can be given. In particular we do not know how *P*. *pluvialis* survives during lull years between outbreaks, nor how it develops and initiates new disease cycles. There is a need to determine what factors lead to high disease severity in some years and if it will be possible to forecast them, in order to target chemical control applications efficiently (though if dependent on particular environmental conditions during the time an epidemic is actually developing, prediction may be problematic).

A number of basic research studies are therefore needed:

- Undertake further studies with greater numbers of exchange plants and spore traps, at several locations and in two or more successive years to increase the likelihood of one being successfully run in an inoculum producing diseased stand, incorporating on-site weather recording, in order to verify and define the pathogen life cycle more precisely in relation to season, ultimately to define a suitable chemical treatment schedule.

- To the same end, conduct a complementary chemical application timing trial once a suitable fungicide is determined.

- Continue with longer term monitoring of the red needle cast in parts of the North Island towards an understanding of factors leading to disease outbreaks, with forecasting as a potential outcome.

- Conduct an individual tree study to learn how *P*. *pluvialis* survives between disease events, and to follow the course of the disease from initial infection.

- Undertake definitive studies in cabinets by inoculating susceptible potted plants with suitable inoculum (quantified standard suspensions of sporangia and zoospores) under a range of controlled environmental conditions to determine environmental and inoculum thresholds for infection.

- Continue the laboratory environmental tolerance studies to define the factors regulating survival periods for sporangia and other propagules of *P*. *pluvialis* as a contributing factor to rate of infection during a disease outbreak.

1 Introduction

Red needle cast is a significant foliage disease causing discoloration and defoliation in stands of *Pinus radiata* D. Don in parts of New Zealand (Dick et al. 2014). Research has been underway since before 2012 to understand how the disease develops and to resolve the life cycle of the causal agent, *Phytophthora pluvialis* Reeser, W.L. Sutton & E.M. Hansen (e.g. Hood et al. 2013a, 2014a; Hood and Uaea 2015; Williams et al. 2016). An awareness of the behaviour of *P. pluvialis* in relation to season and weather variables is paramount to achieving efficient disease management (Rolando et al. 2013; Williams et al. 2016). Unfortunately, uneven patterns in year-to-year symptom expression and severity have hampered this work and it is not yet possible to predict where and when the disease will occur (Kimberley et al. 2016). As a result, two previous studies in this series were run at locations and times when red needle cast failed to appear with any intensity (Hood et al. 2014a, Hood and Uaea 2015). It was therefore decided to conduct the present study in a forest on the east coast of the North Island where past outbreaks had generally been more frequent and severe, increasing the chances of a successful outcome.

Previous work used proxy weather data derived from nearby meteorological stations, supplied by the National Institute of Water and Atmospheric Research (NIWA), in order to make comparisons with disease periodicity. In this new study, recording meteorological stations were set up at the study sites themselves, in order to acquire more precise and relevant local weather data.

2 Materials and methods

2.1. Sites and subsites

The study was run at two sites, 3 km apart, 500m a.s.l., in Wharerata Forest, in hill country south of Gisborne, within stands of *Pinus radiata* where red needle cast had occurred in previous years (Williams et al. 2016). These were at Helen Track off Maxwell Road (Site 1; Cpt. 34/4; planted 2002; New Zealand Transverse Mercator coordinates 2019889/5682890) and Paritu Road (Site 2; Cpt. 32/5; planted 2001; NZTM 2022711/5683656).[1] An additional "subsite" was subsequently established adjacent to each site closer[2] to trees that were becoming diseased and showing red needle cast symptoms (Fig. 1). These subsites were located 500m southwest of Site 1 (Subsite 1; Cpt. 34/6; planted 2004; NZTM 2019697/5682482) and 150m southeast of Site 2 (Subsite 2; Cpt. 32/5; planted 2001; NZTM 2022798/5683527). At the time of the study, stands had been waste thinned to around 600-700 stems/ha with final crop trees pruned to 7 m height.

2.2. Inoculum traps

As in earlier work (Williams et al. 2016), traps for capturing spores/sporangia[3] of *P. pluvialis* released from diseased stand trees were set up to indicate indirectly when inoculum was available for infection of living foliage throughout the study period (Dance et al. 1975). Traps consisted of freshly picked needle fascicles of *P. radiata* held in coarse mesh bags floating on the surface of deionised water, initially in deep plastic trays (cover figure), but for most of the trial in square plastic buckets. Containers were covered in a plastic coated wire grid to exclude litter. Fascicles

[1] Equivalent longitude/latitude coordinates: Site 1: 177.841198°E/38.902488°S; Subsite 1: 177.839241°E/38.906243°S; Site 2: 177.873155°E/38.894258°S; Subsite 2: 177.874234°E /38.895375°S. A red needle cast phosphite spray trial was conducted in part of Helen Track more than 3 years earlier without effect (Rolando et al. 2013). Sites and subsites lay outside the sprayed areas.
[2] About ≈20m, or less, between subsite plants and traps, and nearest diseased stand trees.
[3] It has yet to be established whether detached sporangia, swimming zoospores, or both, predominate as infective *P. pluvialis* propagules. However, observation of discharged sporangia indicate that zoospores play a significant role. See also Footnote 19.

Figure 1. Red needle cast symptoms developing in the lower crowns of trees in two stands where the subsites were set up at Wharerata Forest in May 2016. Above: at Subsite 1 (taken 3 May 2016, four weeks prior to subsite establishment). Below: at Subsite 2 (taken 31 May 2016, at the time when the subsites were initiated).

were taken from a plant of a clone known from detached needle inoculation assays to be receptive to colonisation (Nari Williams, *pers. comm.*), 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 on site the following day. However, due to the loss of this plant, needle baits from 31 May 2016 were taken from a second plant, also amenable to colonisation by *P. pluvialis*[4]. Traps remained in place on the ground throughout the trapping period while needle baits and deionised water were changed at fortnightly intervals.

[4] Needles from a third receptive plant in the Scion nursery were used on 18 May 2016, only.

2.3. Plants

Indicator plants were deployed fortnightly to determine when during the study released spores/sporangia were infecting living foliage. Approximately 100 grafted cuttings of each of two clones (KP1 and KP2) potted individually in 9L plastic buckets were used. The same clones had been utilised in previous experiments and were derived from two trees near Rotorua known to be susceptible to red needle cast (Hood et al. 2014a). Cuttings were taken and grafted onto root stocks in open beds during July 2013, and plants were eventually potted up the following year. In September 2014 plants were placed under cover in the Scion nursery, Rotorua, and watered from below to avoid any risk of natural infection by *P. pluvialis* (Fig. 2, upper). This risk was later considered negligible (red needle cast has not been found infecting plants in the Scion nursery) and in late August 2015 they were moved outside to a better growing environment (Fig. 2, lower). Plants were grouped in their separate clonal blocks at all times in the nursery. Some minor infection by *Dothistroma septosporum* (Dorogin) M. Morelet was observed both before and after plants were placed in and removed from under cover, and browning of older foliage from an unexplained physiological cause, as has occurred previously (Hood et al. 2013a, 2014a, Hood and Uaea 2015), appeared towards the end of the study period, but these did not affect the trial outcome. As in all these studies, no fungicides were ever applied to the experimental plants, though small amounts of general fertiliser were occasionally applied to the soil. Plants were tall when the study commenced and were topped early in August 2015 for convenience in transporting them to and from the field (cover figure; Fig. 2).

Due to decreasing numbers of acceptable stock still available (each plant was deployed in the field only once in each season), six KP2 clonal plants from a younger set (grafted in July 2015 and potted up in May 2016) were used at the very end of the trial. These were held at a separate location in the nursery, when not in the field.

2.4. Meteorological stations

A solar powered, continually recording meteorological station was run at each of the two sites throughout the duration of the trial (Fig. 3). Data recorded at 15 minute intervals were: rainfall (total; mm), air temperature (average; °C), photosynthetically active solar radiation (total; µmoles/m^2/sec.), relative humidity (a point sample; %) and surface wetness as an estimate of free water present on needle surfaces (average electrical resistance; kohms).

2.5. Stand condition

In order to monitor the level of red needle cast, five trees were tagged at each site for routine assessment of disease severity. This was to see if there might be any association in timing between availability of inoculum and the appearance of crown symptoms in the stand trees providing the inoculum. Individual monitoring trees were not set up at the subsites.

2.6. Procedure

2.6.1. Trial period

The trial was conducted over two seasons, between 11 August and 10 December 2015 and between 19 April 2016 and 8 February 2017.

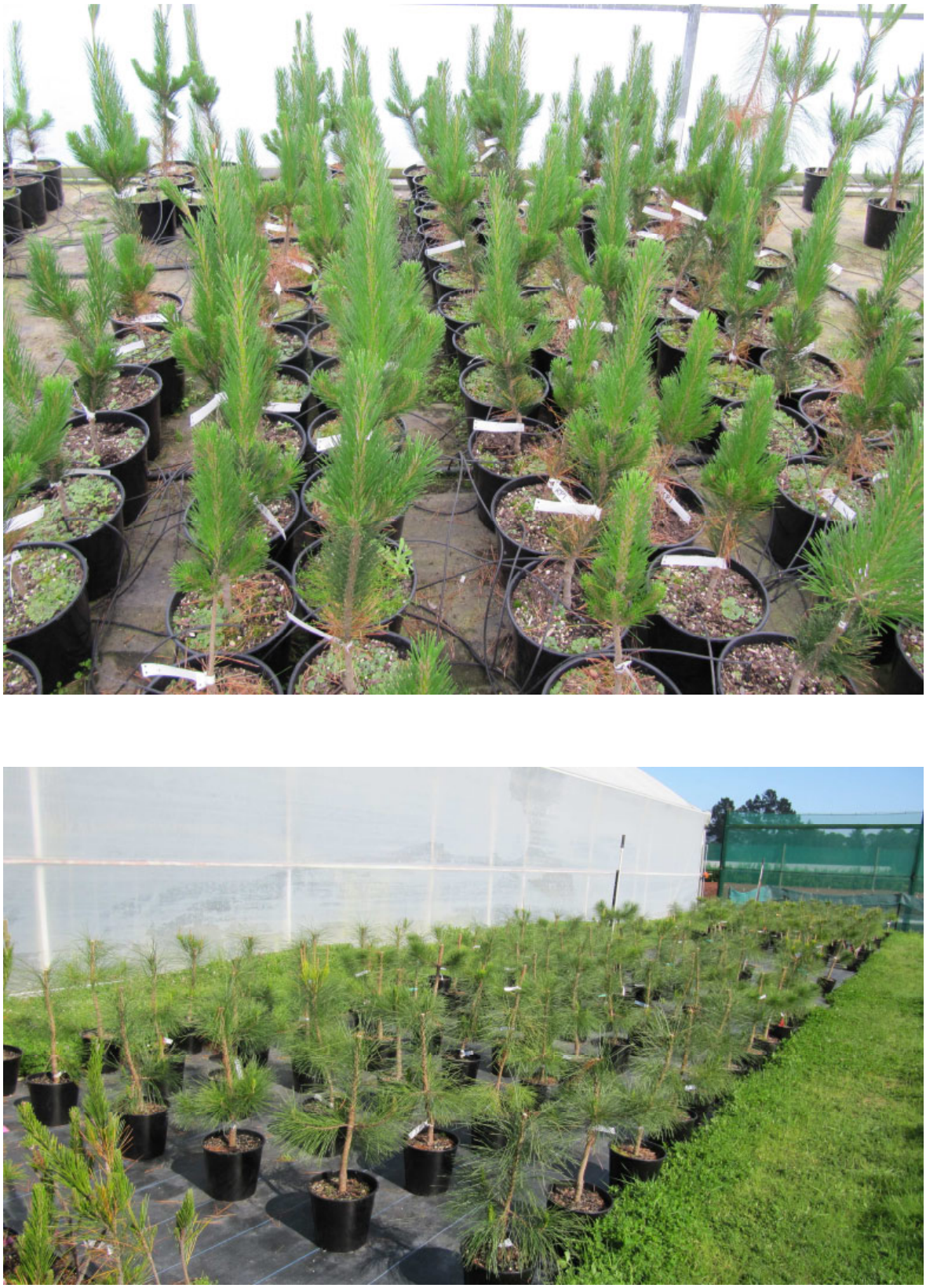

Figure 2. Study plants at Scion nursery. Above: under cover (part of KP1 block, taken 29 October 2014). Below: after moving outside (KP2 block, taken on 7 October 2015; plants are topped).

2.6.2 Monitoring of inoculum

Three inoculum traps were maintained at each of Sites 1 and 2 during the trial periods from 11 August 2015 until 31 May 2016 (Fig. 4, upper). However, between 31 May 2016 and 13 December 2016 one trap from each site was transferred to and held at one of the newly established subsites (Subsite 1 and 2) leaving two traps remaining at each of Site 1 and Site 2 (Fig. 4, lower; trapping ceased after 13 December 2016). Needle baits, consisting of three fascicles per trap, were changed at 14 day intervals throughout the period that inoculum was monitored (except that at the first and last exchanges, baits were held in the field for 23 and 28 days, respectively). Mesh bags were sterilised in bleach, rinsed and air dried between changes.

Baits returned to the laboratory in the polystyrene container were held overnight at 4°C and on the following day were cut into 5mm lengths which were surface sterilised (30sec in 70% ethanol followed by two 30sec rinses in sterile deionised water, patting dry in a clean paper towel). Ten needle fragments from each trap, especially including any from the margins of characteristic *P. pluvialis* lesions if present, were plated onto a selective, amended carrot agar medium[5] and incubated at 17°C. Plates were monitored and any emerging isolates likely to be *P. pluvialis* or *Phytophthora kernoviae* Brasier, Beales & Kirk (the latter species occasionally also being associated with red needle cast-like symptoms) were subcultured on carrot agar[6] to confirm identity morphologically.

Figure **3**. M**eteoro**logical station at Site 2 (taken 29 October 2015).

Positive controls consisted of two additional *P. radiata* needle fascicle baits from the identical source plant floating in a container of sterilised pond water at 4°C in the laboratory over the same fortnight as each set of field baits. Included in the container were several squares cut from a carrot agar culture of *P. pluvialis* (isolate NZFS 4234 in the first season and in the second season, isolate NZFS 4175 until 31 October and NZFS 4268 thereafter; New Zealand Forest Research Institute Culture Collection, Rotorua). The pond water was to induce production of sporangia and release of swimming zoospores for colonisation of bait needles. Negative controls were freshly collected

[5]Carrot agar (footnote 6) with the following antibiotics added after autoclaving: 8mL ampicillin (from frozen 25mg/mL stock), 0.05g nystatin (dissolved in 1-2mL 90% ethanol), 0.01g rifampicin (dissolved in 1-2mL acetone) and 0.4mL pimaricin (from 2.5% aqueous suspension) (cf. Drenth and Sendall, 2001; Eppo, 2013).

[6]100g washed and diced carrot pieces blended in 500mL deionised water, 15g agar added, made up to 1L with deionised water, autoclaved at 121 °C for 15min and agitated during pouring to disperse carrot fragments (Erwin and Ribeiro, 1996).

fascicles processed directly without *P*. *pluvialis* cultures as, and along with, the returned field and the positive control baits. In the first season controls were set up for alternate field samples, only.

Figure 4. Exchange plants and a permanent positive control plant (red ribbon), and inoculum traps, at trial sites. Above: at Site 1 (taken 29 October 2015). Below: at Subsite 1 (taken 31 May 2016, at time of subsite establishment).

2.6.3. Monitoring of infection

Sets of arbitrarily selected infection indicator study plants were placed in and returned from the field in succession throughout the trial period. Plants were positioned in pits dug and lined with plastic film and watered with tap water as necessary during field visits. Due to constraints on numbers, plants deployed in the first season were used again during the following season. In the first season, between 11 August and 10 December 2015, three plants of each clone were placed at each of Sites 1 and 2 during each exchange (12 plants, total; Fig. 4, upper). In the second season, between 19 April and 31 May 2016 two plants of each clone were exchanged systematically at each of these sites (8 plants, total). However, with the establishment of the new subsites during the second season, from 31 May until 13 December 2016 one plant of each clone was positioned at each subsite, with another plant of each clone continuing to be placed at each original site (i.e. still 8 plants, total; Fig. 4 lower).

Plants were changed at 14 day intervals during both seasons, on the same dates as the inoculum bait exchanges. However, at the first exchange they were held for 23 days in the field, and from 15 November 2016 plants were not changed until 13 December (the date when inoculum trapping ceased; an interval of 28 days). Infection monitoring then ceased at Sites 1 and 2 and Subsite 2. Subsite 1 (Helen Track) was maintained, with two more 28-day changes but using three of the younger KP2 plants at each exchange, until 8 February 2017. Apart from these younger plants, all those returned from the field were replaced at arbitrary locations within each of their respective clonal blocks.

Positive controls consisted of three (first season; 12, total) or two (second season; 8, total) plants of each clone placed permanently at each site throughout the full length of each monitoring period. Except that, at the time of the establishment of the new subsites during the second season, one control plant of each clone was moved from each site to its respective subsite, leaving one of each clone (two plants) remaining at each site. All controls were free of red needle cast symptoms at this time (as were all exchange plants when placed in the field). In the second season, controls were returned from the field on 10 January 2017 (Sites 1 and 2, Subsite 2) and on 8 February 2017 (Subsite 1). Negative controls consisted of plants retained in the nursery throughout the full period during each season.

Plants were monitored in both field and nursery and assessed at intervals during each season. Plant health was evaluated according to the criteria in Table 1.

Table 1: Scale for assessing health of potted cuttings.

0	Healthy; needles fresh, green (0% foliage affected)
1	Some new yellowing (or browning), discoloration (1-10% foliage affected)
2	Moderate new yellowing, discoloration (11-50% foliage affected)
3	Much new yellowing, discoloration (51-100% foliage affected)

Foliage on each plant was also inspected periodically for symptoms of infection by *P*. *pluvialis* or *P*. *kernoviae* (a dull, khaki-green discoloration along a short, discrete segment length of the needle, frequently accompanied by small, dark, resinous spots or narrow black bands). Symptomatic needles were examined in the laboratory for confirmation of infection by either of these species. This was done by using a commercial ImmunoStrip kit (Agdia Inc., Elkhart, Indiana, USA) to indicate the presence of a phytophthora (i.e. identification to genus), followed by sequencing from the ImmunoStrip or by morphological examination of sporangia present on the needle surface on or close to the lesion, to identify species.

2.6.4. Monitoring of stand condition

The upper and lower crown of each of the tagged trees at Site 1 and Site 2 was assessed for defoliation and discoloration during each fortnightly field visit according to the scales shown in Table 2. Assessments were undertaken individually on different dates by four separate observers.

Table 2: Assessment scales for the upper and lower crown on each stand tree for crown defoliation ("transparency", "density", "gappiness") and discoloration (yellowing or browning).

Defoliation		Discoloration	
0	0% (fully foliated)	0	0% (fully green)
1	1-25%	1	1-25%
2	26-50%	2	26-50%
3	51-75%	3	51-75%
4	76-100% (heavily defoliated)	4	76-100% (extensive discoloration)

Figure 5. Assessment tree No. 4 at Site 1. When taken (29 October, 2015), this healthy tree scored 0 and 0-1 for defoliation and discoloration, respectively, in the lower crown, and 0 for both attributes in the upper crown. Trees at Sites 1 and 2 remained free of red needle cast throughout the trial period, whereas crown symptoms of the disease developed from May 2016 during the second season at the two subsites (Fig. 1).

2.6.5. Weather data

Bar graphs were prepared for each site showing, for weekly intervals throughout the study period, total rainfall, average temperature (means of each 15 minute average), average relative humidity (means of each 15 minute sample record), total solar radiation and surface wetness period (sum of the 15 minute intervals recorded as wet). Surface wetness was categorised as wet (resistance, 0-150 kohms) or slightly wet (resistance,150-7999 kohms), following the manufacturer's recommendation.

Plots were inspected for any trends in weather variables in relation to inoculum availability and infection period data.

3. Results

3.1. Inoculum availability

Results for *P. pluvialis* are shown in Table 3. Inoculum of *P. pluvialis* was not detected by trapping during the first season (Table 3a). In the second season inoculum was confirmed as available at the two subsites on 18 October, but on no other dates, and never at the two original sites (Table 3b). Positive controls in the laboratory showed variable results, but generally confirmed the reliability of the technique. However, between 21 September and 13 December in the second season positive control baits failed to detect *P. pluvialis* inoculum (Table 3b). *P. pluvialis* inoculum was not detected in any of the negative controls (Table 3).

Inoculum of *P. kernoviae* was not detected in inoculum traps throughout the course of the trial. There was also no evidence of this species in positive and negative control baits (positive controls were exposed to zoospores only of *P. pluvialis*).

3.2. Infection period

Infection by *P. pluvialis* did not occur during the first season on any of the infection indicator plants, including the positive field and negative nursery controls (Table 4a).

In the second season, infection by *P. pluvialis* occurred on fortnightly exposed exchange plants placed at Subsite 1 on 29 July (KP2 clone), 23 August (KP2), 21 September (KP1) and 18 October (KP1) (Table 4b; Figs. 6, 7). Infection was not detected on any exchange plants placed at Subsite 2 or Sites 1 and 2. Infection by *P. pluvialis* occurred on all positive control plants of both host clones held at both Subsites 1 and 2 (Table 4b; Fig. 8). Infection was not detected on control plants at either of the original Sites 1 and 2, except that on one plant at Site 1, two symptomatic needles were found infected by a *Phytophthora* species that was likely to have been *P. pluvialis*, but there was insufficient material available for this to be confirmed (Table 4b). Negative control plants held permanently in the nursery were not infected. When symptoms occurred, they were generally present on the older foliage lower on the plant, though because they had been topped it was not clear if these needles were still less than one year old (Fig. 8).

Phytophthora kernoviae was not detected infecting any of the study plants throughout the course of the trial.

Table 3: Detection of *P. pluvialis* inoculum in pine needle baited traps set at successive fortnightly intervals for periods of 14 days. Nº. needle fragments colonised (out of Nº. plated, in brackets; data from traps at each site combined).

(a) First season (2015)

Date placed in field	11 Aug.[1]	3 Sep.	17 Sep.	1 Oct.	15 Oct.	29 Oct.	12 Nov.	26 Nov.
Site								
1 (3 traps)	0 (30)	0 (30)	0 (30)	0 (30)	0 (30)	0 (30)	0 (30)	0 (30)
2 (3 traps)	0 (30)	0 (30)	0 (30)	0 (30)	0 (30)	0 (30)	0 (30)	0 (30)
Positive control	9 (10)	-	2 (10)	-	1 (10)	-	0 (10)	-
Negative control	0 (10)	-	0 (10)	-	0 (10)	-	0 (10)	-

(b) Second season (2016-17; continues on to next page)

Date placed in field	19 Apr.	3 May	18 May	31 May	14 Jun.	29 Jun.	13 Jul.	26 Jul.
Site/subsite[2]								
Site 1 (2 traps)	0 (30)	0 (30)	0 (30)	0 (20)	0 (20)	0 (20)	0 (20)	0 (20)
Subsite 1 (1 trap)	-	-	-	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)
Site 2 (2 traps)	0 (30)	0 (30)	0 (30)	0 (20)	0 (20)	0 (20)	0 (20)	0 (20)
Subsite 2 (1 trap)	-	-	-	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)
Positive control	5 (10)	3 (10)	7 (10)	-[3]	7 (10)	10 (10)	10 (10)	10 (10)
Negative control	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)

Table 3 concluded on next page.

Table 3: (concluded)

(b) Second season (2016-17), continued from previous page

Date placed in field	9 Aug.	23 Aug.	7 Sep.	21 Sep.	4 Oct.	18 Oct.	1 Nov.	15 Nov.[4]
Site/subsite								
Site 1 (2 traps)	0 (20)	0 (20)	0 (20)	0 (20)	0 (20)	0 (20)	0 (20)	0 (20)
Subsite 1 (1 trap)	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)	2 (10)	0 (10)	0 (10)
Site 2 (2 traps)	0 (20)	0 (20)	0 (20)	0 (20)	0 (20)	0 (20)	0 (20)	0 (20)
Subsite 2 (1 trap)	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)	1 (10)	0 (10)	0 (10)
Positive control	10 (10)	7 (10)	10 (10)	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)
Negative control	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)	0 (10)

[1]Held in field for 23 days.
[2]Subsites were established 31 May. Prior to this there were 3 traps at each original site.
[3]Needles dried over a long weekend.
[4]Held in field for 28 days.

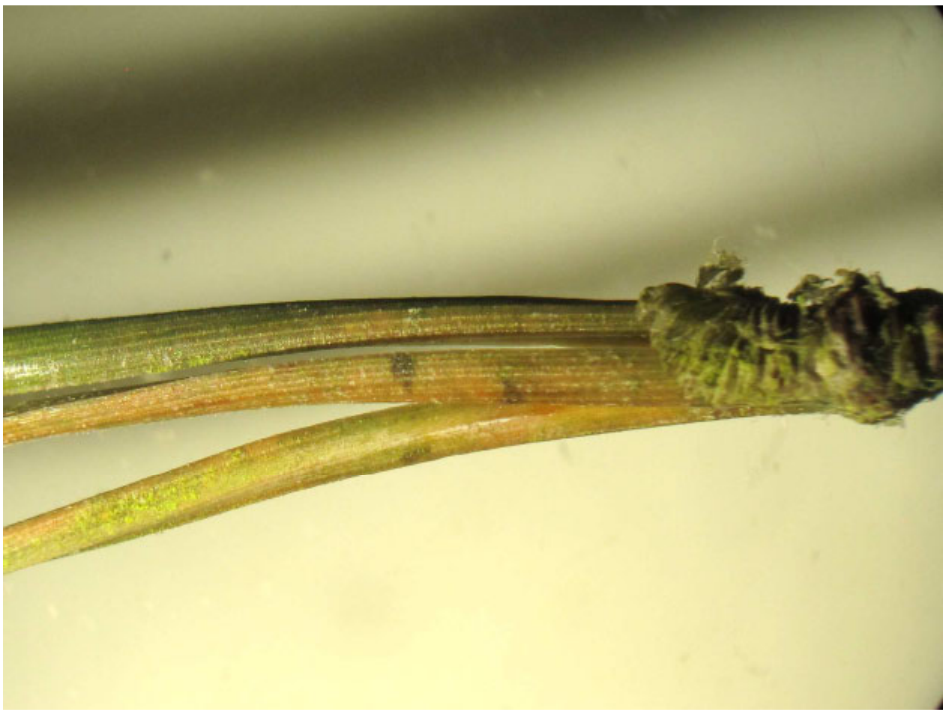

Figure 6. Symptoms of red needle cast infection at base of a needle fascicle from exchange plant N$^{o.}$ KP2-14 exposed for 14 days at Subsite 1 between 29 June and 13 July, 2016.

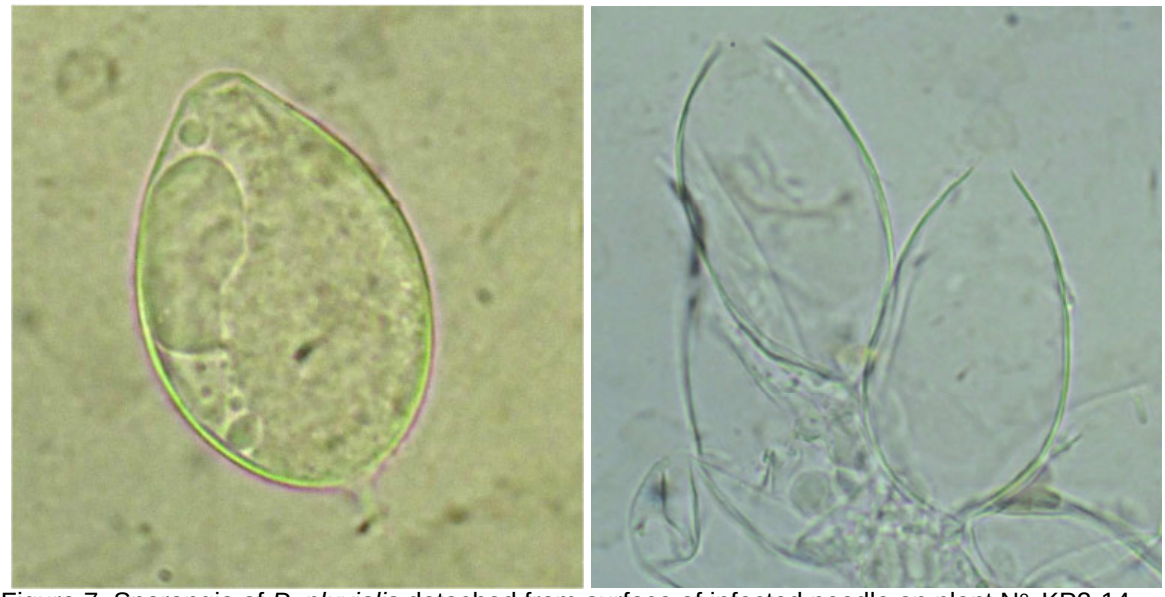

Figure 7. Sporangia of *P*. *pluvialis* detached from surface of infected needle on plant Nº. KP2-14. Left: undischarged, zoospores not yet formed. Right: empty of zoospores.

Table 4: Occurrence of infection by *P*. *pluvialis* on one or more needles of potted radiata pine grafted cuttings of two clones exposed in the field at fortnightly intervals for periods of 14 days. Nº. plants infected (out of Nº. placed, in brackets).

(a) First season (2015)

Date placed in field		11 Aug.[1]	3 Sep.	17 Sep.	1 Oct.	15 Oct.	29 Oct.	12 Nov.	26 Nov.
Site	Clone								
1	KP1	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)
	KP2	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)
2	KP1	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)
	KP2	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)
1 (Positive control)[2]	KP1	0 (3)							
	KP2	0 (3)							
2 (Positive control)[2]	KP1	0 (3)							
	KP2	0 (3)							
Nursery (Negative control)	KP1	0 (6)							
	KP2	0 (6)							

[1]First set of exchange plants held in field for 23 days.
[2]Positive control plants held in field for 126 days until 15 December.

Table 4 continued on next page.

Table 4: (continued)

(b) Second season (2016-17; continues on to next page)

Date placed in field		19 Apr.	3 May	18 May	31 May	14 Jun.	29 Jun.	13 Jul.	26 Jul.	9 Aug.
Site/Sub-site[3]	Clone									
Site 1	KP1	0 (2)	0 (2)	0 (2)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
	KP2	0 (2)	0 (2)	0 (2)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Subsite 1	KP1	-	-	-	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
	KP2	-	-	-	0 (1)	0 (1)	1 (1) *†‡	0 (1)	0 (1)	0 (1)
Site 2	KP1	0 (2)	0 (2)	0 (2)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
	KP2	0 (2)	0 (2)	0 (2)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Subsite 2	KP1	-	-	-	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
	KP2	-	-	-	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Site 1 (Positive control) [4]	KP1	0 (2)			0 (1)					
	KP2	0 (2)			1 (1) * 21 Sept. (only 2 needles)					
Subsite 1 (Positive control) [4]	KP1	-			1 (1) *†‡ 21 Sept., 15 Nov. (>10 needles)					
	KP2	-			1 (1) *†‡ 21 Sept., 15 Nov. (>10 needles)					
Site 2 (Positive control) [4]	KP1	0 (2)			0 (1)					
	KP2	0 (2)			0 (1)					
Subsite 2 (Positive control) [4]	KP1	-			1 (1) *†‡ 21 Sept., 15 Nov. (>10 needles)					
	KP2	-			1 (1) *†‡ 21 Sept., 15 Nov. (>10 needles)					
Nursery (Negative control)	KP1	0 (ca. 20)								
	KP2	0 (ca. 20)								

For positive controls, dates and approximate numbers of needles per plant on which infection was found are shown.

Infection by *P. pluvialis* confirmed by ImmunoStrip positive to genus (*); and to species by sequencing (†) and/or morphology of sporangia from needle surface (‡).

[3]Subsites established 31 May. [4]One control plant of each clone transferred from each original site when subsites established; controls maintained in field until 10 Jan. (or 8 Feb., Subsite 1) 2017.

Table 4 concluded on next page.

Table 4: (concluded)

(b) Second season (2016-17), continued from previous page

Date placed in field		23 Aug.	7 Sep.	21 Sep.	4 Oct.	18 Oct.	1 Nov.	15 Nov.[5]	13 Dec.[5]	10 Jan.[5]
Site/Sub-site[3]	Clone									
Site 1	KP1	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	-	-
	KP2	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	-	-
Subsite 1	KP1	0 (1)	0 (1)	1 (1) *‡	0 (1)	1 (1) *†‡	0 (1)	0 (1)	-	-
	KP2	1 (1) *‡	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (3)[6]	0 (3)[6]
Site 2	KP1	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	-	-
	KP2	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	-	-
Subsite 2	KP1	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	-	-
	KP2	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	-	-

[3]Subsites were established 31 May.

[5]Exchange plants held in field for 28 days.

[6]A younger set of KP2 clonal plants.

Infection by *P. pluvialis* confirmed by ImmunoStrip positive to genus (*); and to species by sequencing (†) and/or morphology of sporangia from needle surface (‡).

Figure 8. Positive control plant No. KP1-2 held permanently at Subsite 1, as it appeared on 7 September 2016, with lower foliage showing symptoms of red needle cast following infection by *P. pluvialis* (compare with condition on earlier view of same plant shown in foreground in Fig. 4, lower). Left: general view of whole plant. Right: closer view of diseased needles.

Results of assessments of plant health are shown in Table 5. Plants generally remained healthy throughout the majority of the trial period, with mean scores ranging mostly ≤ 1 (Table 5); i.e. plants had foliage that was typically either green or with not more than ca. 10% discoloration, on average (Table 1). Although not verified statistically, no conspicuous differences were noticeable between sites or subsites, clones, and field exposed or not exposed plants over time (Table 5). Where controls were not assessed they also did not stand out from the other plants during periodic inspections. The numbers of needles and plants that became infected at the subsites in the second season were too small to influence the overall averages (cf. Fig. 8). However, in early November 2016, subsequent to the last assessment, extensive unexplained browning of older foliage appeared on many of the plants in the nursery, including both those that had been exposed to red needle cast in the field as well as others that had not (Fig. 9). Although this reduced the number of exchange plants available, because it occurred near the end of the study the trial outcome was unaffected.

Table 5: Mean assessment score (with standard deviation) for health of *n* study plants by location, plant type and clone.[1, 2]

Location	Plant type	Clone	Date assessed											
			First season, 2015								Second season, 2016			
			15/22 Sep.				19/29 Oct.				22 Dec.			
Site 1	Exposed in field[3]	KP1	6	0.08	(0.20)		18	0.14	(0.23)		23	0.35	(0.28)	
		KP2	6	0.08	(0.20)		18	0.19	(0.25)		24	0.50	(0.30)	
	Positive field control	KP1	-	-	-		3	0.00	(0.00)		3	0.17	(0.29)	
		KP2	-	-	-		3	0.00	(0.00)		3	0.50	(0.00)	
	Negative nursery control	KP1	3	0.17	(0.29)		3	0.17	(0.29)		3	0.50	(0.00)	
		KP2	3	0.00	(0.00)		3	0.29	(0.76)		3	1.67	(1.26)	
Subsite 1	Exposed in field	KP1	-	-	-		-	-	-		-	-	-	
		KP2	-	-	-		-	-	-		-	-	-	
Site 2	Exposed in field[3]	KP1	6	0.08	(0.20)		18	0.19	(0.25)		22	0.39	(0.22)	
		KP2	6	0.08	(0.20)		18	0.36	(0.41)		24	0.58	(0.50)	
	Positive field control	KP1	-	-	-		3	0.50	(0.00)		3	0.67	(0.58)	
		KP2	-	-	-		2	0.50	(0.00)		3	0.67	(0.29)	
	Negative nursery control	KP1	3	0.00	(0.00)		3	0.17	(0.29)		3	0.75	(1.50)	
		KP2	3	0.17	(0.29)		3	1.17	(0.76)		3	0.35	(0.87)	
Subsite 2	Exposed in field	KP1	-	-	-		-	-	-		-	-	-	
		KP2	-	-	-		-	-	-		-	-	-	

Location	Plant type	Clone	Second season, 2016
			27 Sep.
Site 1	Exposed in field[3]	KP1	14 1.00 (0.60)
		KP2	14 0.75 (0.55)
	Positive field control	KP1	- - -
		KP2	- - -
	Negative nursery control	KP1	- - -
		KP2	- - -
Subsite 1	Exposed in field	KP1	8 1.31 (0.53)
		KP2	8 0.75 (0.54)
Site 2	Exposed in field[3]	KP1	14 0.57 (0.27)
		KP2	14 0.86 (0.75)
	Positive field control	KP1	- - -
		KP2	- - -
	Negative nursery control	KP1	- - -
		KP2	- - -
Subsite 2	Exposed in field	KP1	8 1.00 (0.60)
		KP2	8 0.75 (0.54)

[1]Positive control field plants not assessed in September 2015; all controls, including those of subsites, not assessed in September 2016.

[2]Plants that were scored between integer categories (Table 1) allocated to nearest 0.5 value prior to analysis (e.g. a plant scored between 0 and1, treated as 0.5; between 1 and 2, as1.5).

[3]Numbers increase with time as a greater aggregate of exchange plants have become field exposed.

Figure 9. Unexplained browning of older foliage on study plants in the nursery towards the end of the trial (taken 30 November 2016).

3.3. Stand condition

Results of assessments of monitoring trees at the two sites are shown in Table 6. Although inspection indicates some slight unevenness in values between dates (i.e. between different observers), data confirm that trees remained healthy and free of red needle cast at Sites 1 and 2 throughout the trial period (Fig. 5; some of the defoliation is attributable to minor wind stress at these moderately exposed sites, and there was negligible infection by *D*. *septosporum* on the foliage of a number of trees). This situation is in contrast to that at the two subsites where red needle cast developed from May during the second season, only (Fig. 1; trees at the subsites remained healthy during the first season). Although, no monitoring trees were set up to quantify the disease at the two subsites, levels could be classed from general observation as “moderate-to-severe” at Subsite 1 and “slight-to-moderate” at Subsite 2. At Subsite 2, only a few trees were affected by red needle cast, whereas at Subsite 1 the general cluster of diseased trees occupied a significantly greater area.

Table 6: Stand health at each site at successive intervals during the course trial. Means (with standard deviation in brackets) of 5[1] monitoring trees per site[2].

(a) First season (2015)

Site	Category	Crown position	11 Aug.	3 Sep.	17 Sep.	1 Oct.	15 Oct.	29 Oct.	12 Nov.	26 Nov.	10 Dec.	15 Dec.
1	Defoliation	Upper	0.0 (0.0)	1.2 (0.5)	0.4 (0.6)	0.6 (0.6)	1.0 (0.0)	0.0 (0.0)	1.0 (0.7)	0.8 (0.5)	0.6 (0.6)	0.0 (0.0)
		Lower	0.4 (0.6)	1.6 (0.0)	1.2 (0.5)	1.0 (0.0)	1.0 (0.0)	0.0 (0.0)	1.6 (0.6)	0.8 (0.5)	1.0 (0.0)	0.3 (0.5)
	Discoloration	Upper	0.4 (0.6)	1.0 (0.0)	0.2 (0.5)	0.8 (0.5)	1.0 (0.0)	0.0 (0.0)	0.8 (0.5)	0.6 (0.6)	0.4 (0.6)	0.0 (0.0)
		Lower	1.0 (0.0)	1.0 (0.6)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	0.1 (0.3)	1.0 (0.0)	0.4 (0.6)	0.6 (0.6)	0.8 (0.5)
2	Defoliation	Upper	0.0 (0.0)	1.2 (0.5)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	0.2 (0.5)	1.8 (0.5)	0.6 (0.6)	0.4 (0.6)	0.2 (0.5)
		Lower	0.0 (0.0)	1.6 (0.6)	1.4 (0.6)	1.0 (0.0)	1.0 (0.0)	0.2 (0.5)	1.8 (0.5)	1.0 (0.0)	0.6 (0.6)	0.6 (0.6)
	Discoloration	Upper	0.6 (0.6)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	0.0 (0.0)	1.0 (0.0)	1.0 (0.0)	0.6 (0.6)	0.0 (0.0)
		Lower	0.6 (0.6)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	0.0 (0.0)	1.0 (0.0)	0.6 (0.6)	0.6 (0.6)	0.0 (0.0)

(b) Second season (2016-17; continues on to next page)

Site	Category	Crown position	19 Apr.	3 May	18 May	31 May	14 Jun.	29 Jun.	13 Jul.	26 Jul.	9 Aug.	23 Aug.
1	Defoliation	Upper	0.2 (0.5)	0.4 (0.6)	0.1 (0.2)	0.4 (0.4)	0.2 (0.3)	1.0 (0.0)	1.0 (0.7)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
		Lower	0.6 (0.6)	0.4 (0.6)	0.4 (0.2)	0.5 (0.0)	0.2 (0.3)	1.2 (0.5)	1.2 (0.5)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
	Discoloration	Upper	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.2)	0.2 (0.3)	1.0 (0.0)	0.6 (0.6)	0.6 (0.6)	0.2 (0.5)	0.2 (0.5)
		Lower	0.0 (0.0)	0.2 (0.5)	0.1 (0.2)	0.2 (0.3)	0.0 (0.0)	1.2 (0.5)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	0.8 (0.5)
2	Defoliation	Upper	0.0 (0.0)	0.4 (0.6)	0.5 (0.0)	0.7 (0.3)	0.5 (0.0)	0.4 (0.6)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
		Lower	0.2 (0.5)	0.0 (0.0)	0.5 (0.0)	0.7 (0.3)	0.4 (0.2)	1.2 (0.5)	1.0 (0.0)	0.8 (0.5)	1.0 (0.0)	1.0 (0.0)
	Discoloration	Upper	0.4 (0.6)	0.8 (0.5)	0.1 (0.2)	0.2 (0.5)	0.2 (0.5)	0.6 (0.6)	0.0 (0.0)	0.4 (0.6)	0.0 (0.0)	0.0 (0.0)
		Lower	0.0 (0.0)	0.4 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.8 (0.5)	0.0 (0.0)	0.2 (0.5)	0.0 (0.0)	0.2 (0.5)

Table 6 concluded on next page.

[1]Rarely n=4 if one of the trees was not located by an observer.

[2]Plants that were scored between 0 and 1 (Table 2) were allocated a value of 0.5 for analyses.

Table 6: (concluded)

(b) Second season (2016-17), continued from previous page

Site	Category	Crown position	7 Sep.	21 Sep.	4 Oct.	18 Oct.	1 Nov.	15 Nov.	13 Dec.	10 Jan.	8 Feb.
1	Defoliation	Upper	0.2 (0.5)	0.0 (0.0)	0.7 (0.5)	0.4 (0.6)	0.2 (0.5)	0.2 (0.5)	0.2 (0.5)	0.0 (0.0)	
		Lower	0.4 (0.6)	0.0 (0.0)	1.0 (0.0)	1.0 (0.7)	0.8 (0.5)	0.6 (0.6)	0.6 (0.6)	0.2 (0.5)	
	Discoloration	Upper	0.0 (0.0)	0.0 (0.0)	1.0 (0.0)	0.6 (0.6)	0.6 (0.6)	0.0 (0.0)	0.8 (0.5)	0.3 (0.5)	
		Lower	0.0 (0.0)	0.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	0.2 (0.5)	1.0 (0.0)	0.6 (0.6)	
2	Defoliation	Upper	1.0 (0.0)	0.2 (0.5)	1.0 (0.0)	0.2 (0.5)	0.0 (0.0)	0.6 (0.6)	0.0 (0.0)	0.0 (0.0)	
		Lower	1.0 (0.0)	0.0 (0.0)	0.6 (0.5)	0.2 (0.5)	0.2 (0.5)	0.8 (0.5)	0.4 (0.6)	0.2 (0.5)	
	Discoloration	Upper	0.0 (0.0)	0.0 (0.0)	0.2 (0.5)	0.3 (0.5)	0.4 (0.6)	0.0 (0.0)	0.4 (0.6)	0.4 (0.6)	
		Lower	0.0 (0.0)	0.2 (0.5)	0.6 (0.6)	0.5 (0.5)	0.2 (0.5)	0.0 (0.0)	0.8 (0.5)	0.2 (0.5)	

3.4. Meteorological data and infection

Summary graphs of the weather variables recorded at the two study sites are shown for the second season in Figs. 10-15 and for the whole trial period in App. A. A loose correspondence was noticeable between periods of rainfall (Figs. 10, 11) and of surface wetness (Fig. 12), but there were also weeks of significant surface wetness when rainfall was lower. Surface wetness tended to last slightly longer at Site 1 than at Site 2 (Fig. 12). Temperature (Figs. 13, 14) and solar radiation (Fig. 15) were both lower from late autumn to early spring, whereas relative humidity showed no obvious seasonal trend (Fig, 13). However, relative humidity at Site 1 tended to track slightly higher than at exposed Site 2 (Fig. 13).

Infection was detected on exchange plants exposed at the subsites after 31 May 2016 during three of four fortnightly periods of higher than average, more sustained rainfall (Figs. 10, 11). However, infection also occurred on one occasion in early August when rainfall was lower. With these exceptions, infection occurred when weekly rainfall was at least 100mm over an interval of 3 days in the week and there were around 70 hours or more of surface wetness per week. Infection occurred during the cooler part of the year when average and average minimum temperatures were generally not more than 12 °C and 7 °C, respectively (Figs. 13, 14), and photosynthetic radiation was mostly less than 100mole/m^2/week beneath the canopy (in the vicinity of the exchange plants and traps; Fig. 15).

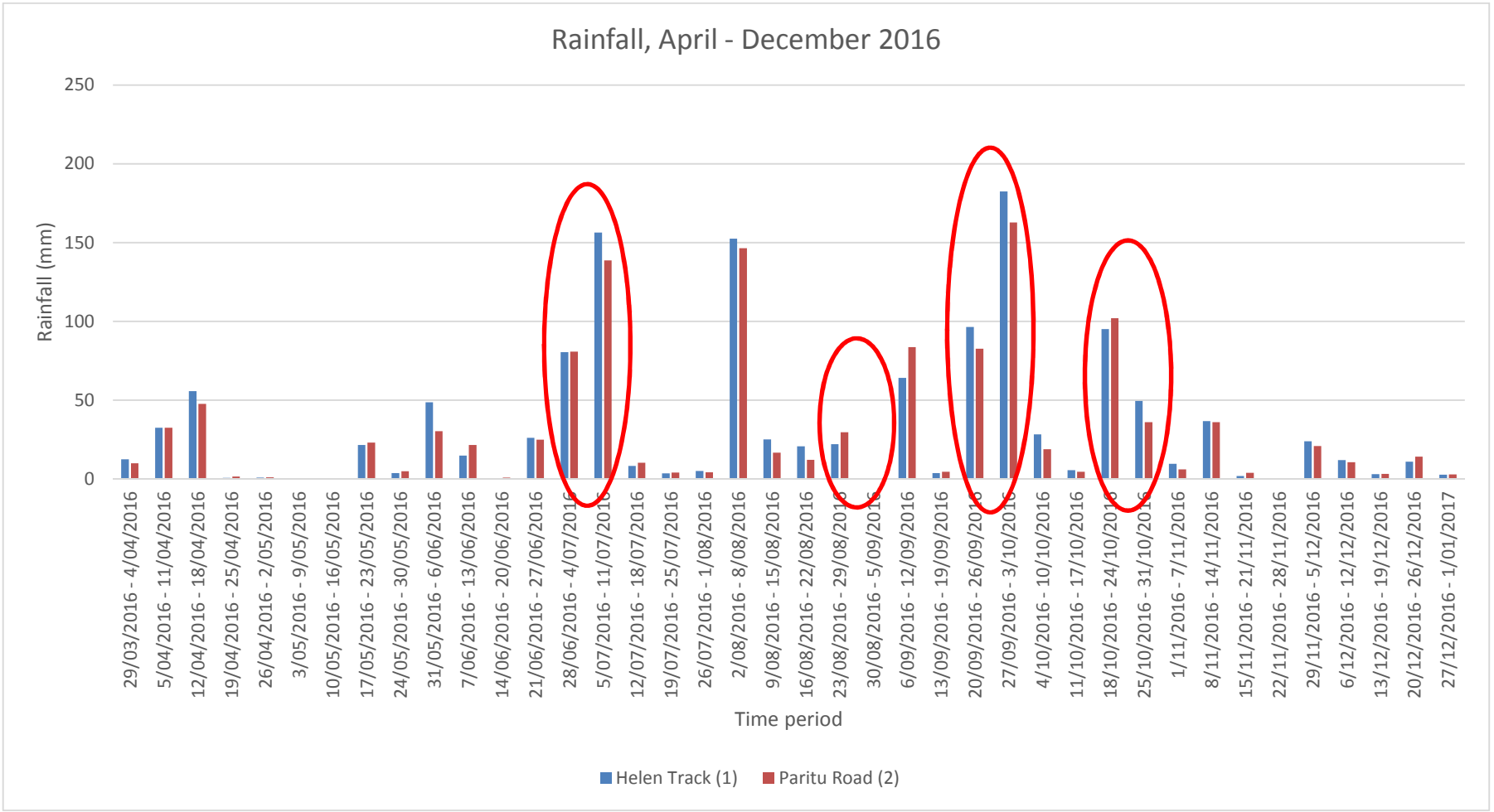

Figure 10. Total weekly rainfall during the second season at Site1 (Helen Track) and Site 2 (Paritu Road). The red circles indicate the quantities of rainfall that occurred during the two-week exposure intervals in which infection was detected on exchange plants at nearby subsites (Table 4).

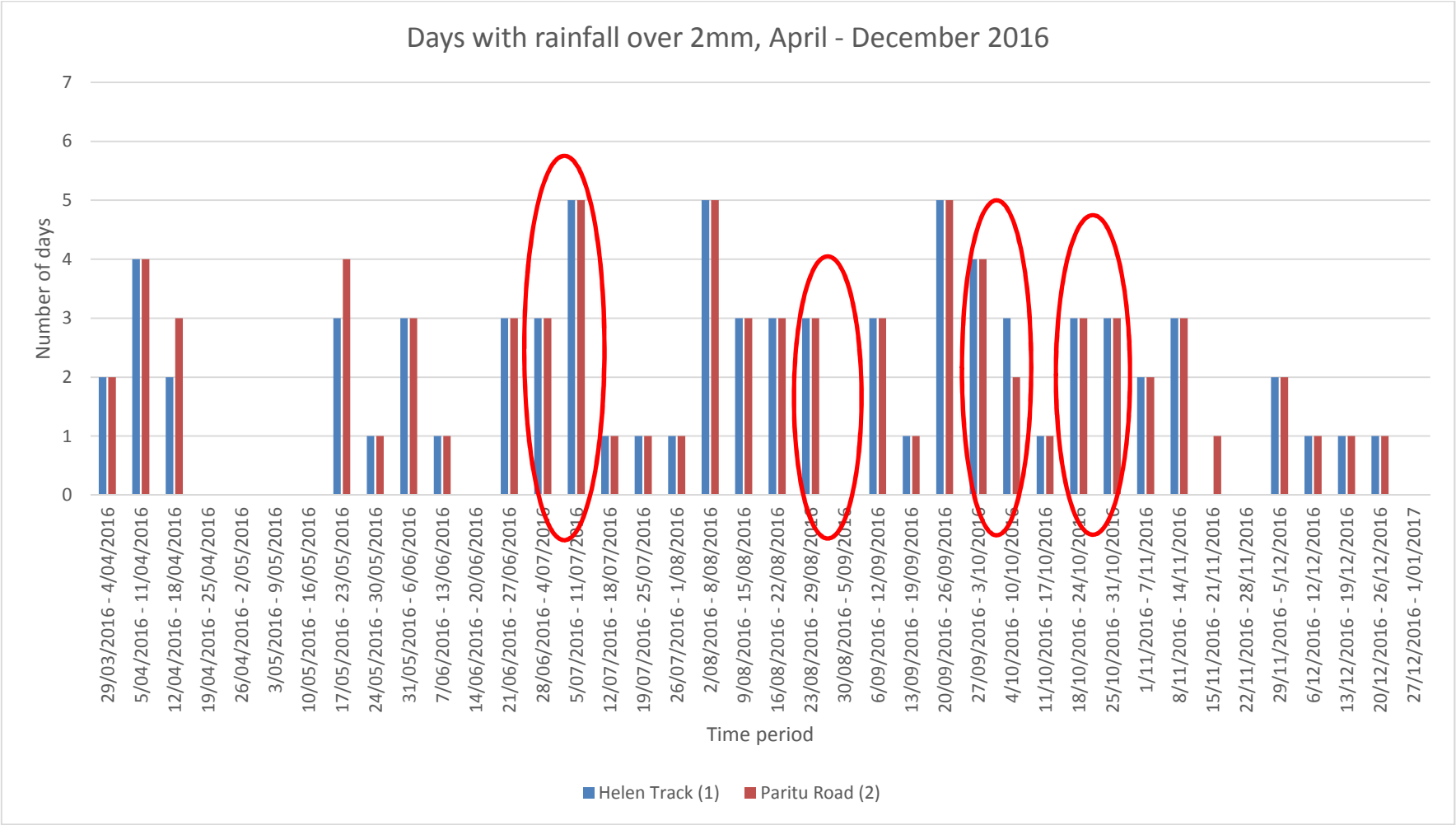

Figure 11. Number of days per week with rainfall greater than 2mm during the second season at Site1 (Helen Track) and Site 2 (Paritu Road). The red circles indicate the numbers of rain days that occurred during the two-week exposure intervals in which infection was detected on exchange plants at nearby subsites (Table 4).

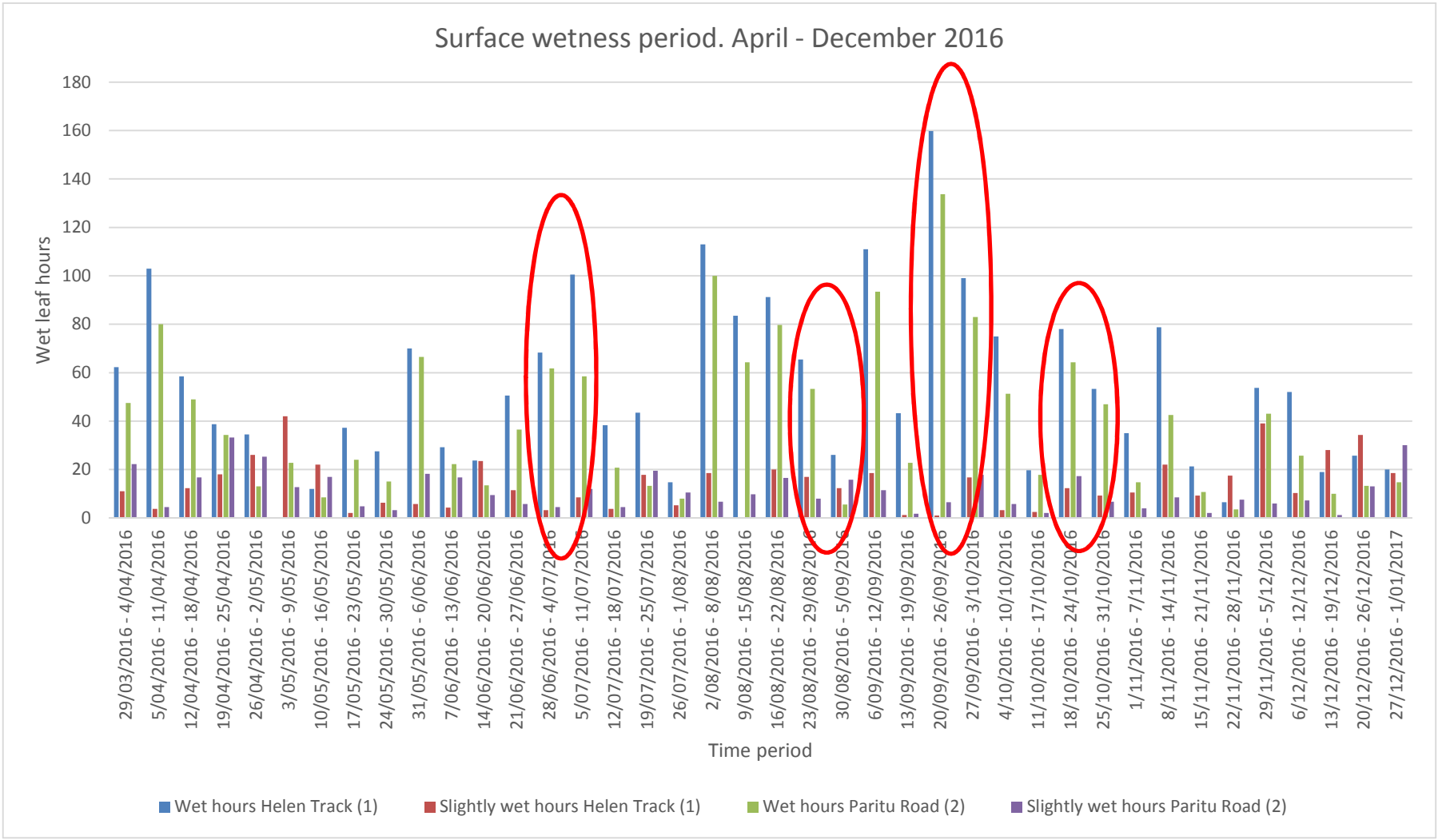

Figure 12. Weekly surface wetness period during the second season at Site1 (Helen Track) and Site 2 (Paritu Road). The red circles indicate the surface wetness periods that occurred during the two-week exposure intervals in which infection was detected on exchange plants at nearby subsites (Table 4).

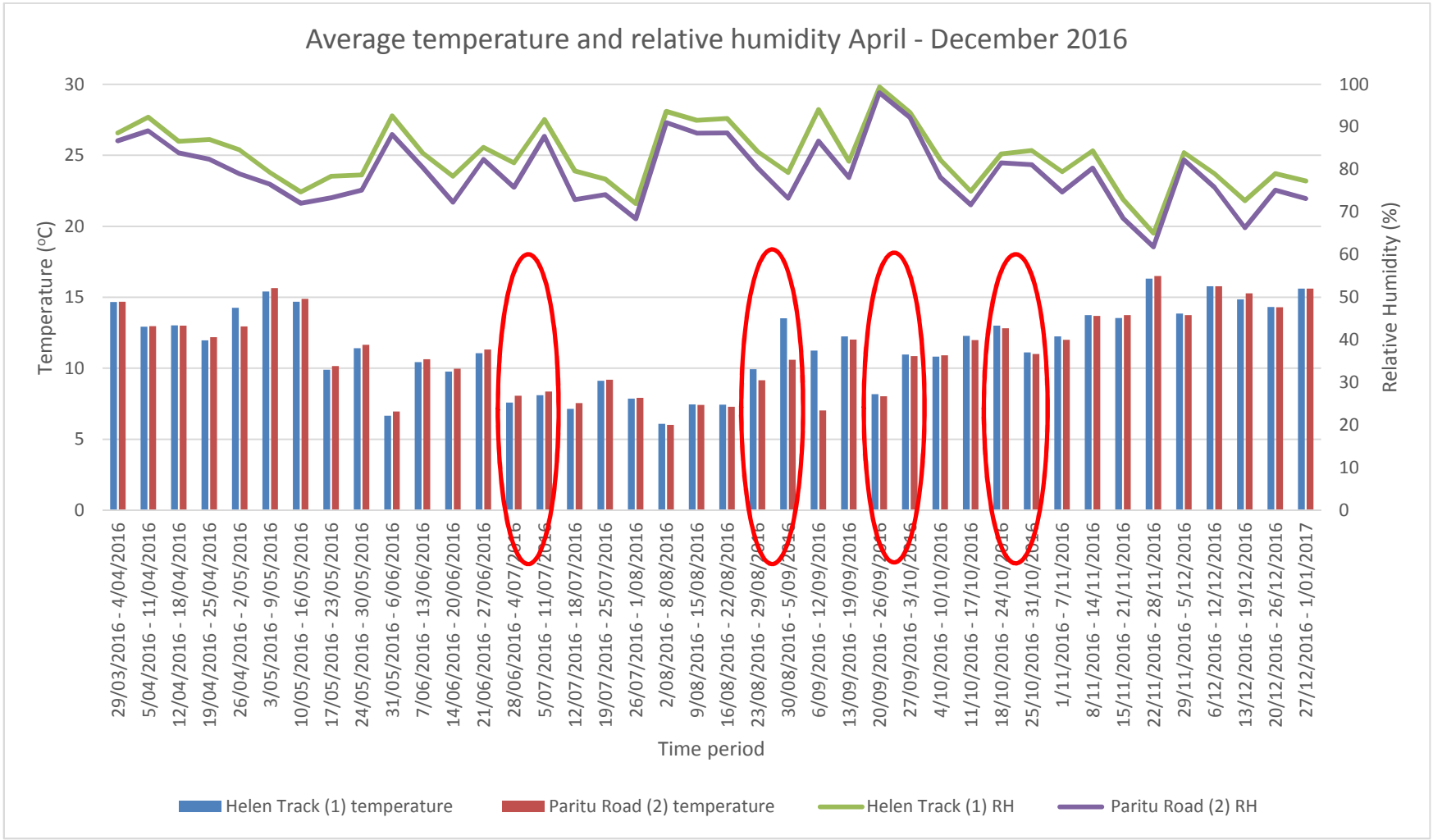

Figure 13. Average weekly temperature and relative humidity (means of daily means) during the second season at Site1 (Helen Track) and Site 2 (Paritu Road). The red circles indicate the average temperatures and relative humidities that occurred during the two-week exposure intervals in which infection was detected on exchange plants at nearby subsites (Table 4).

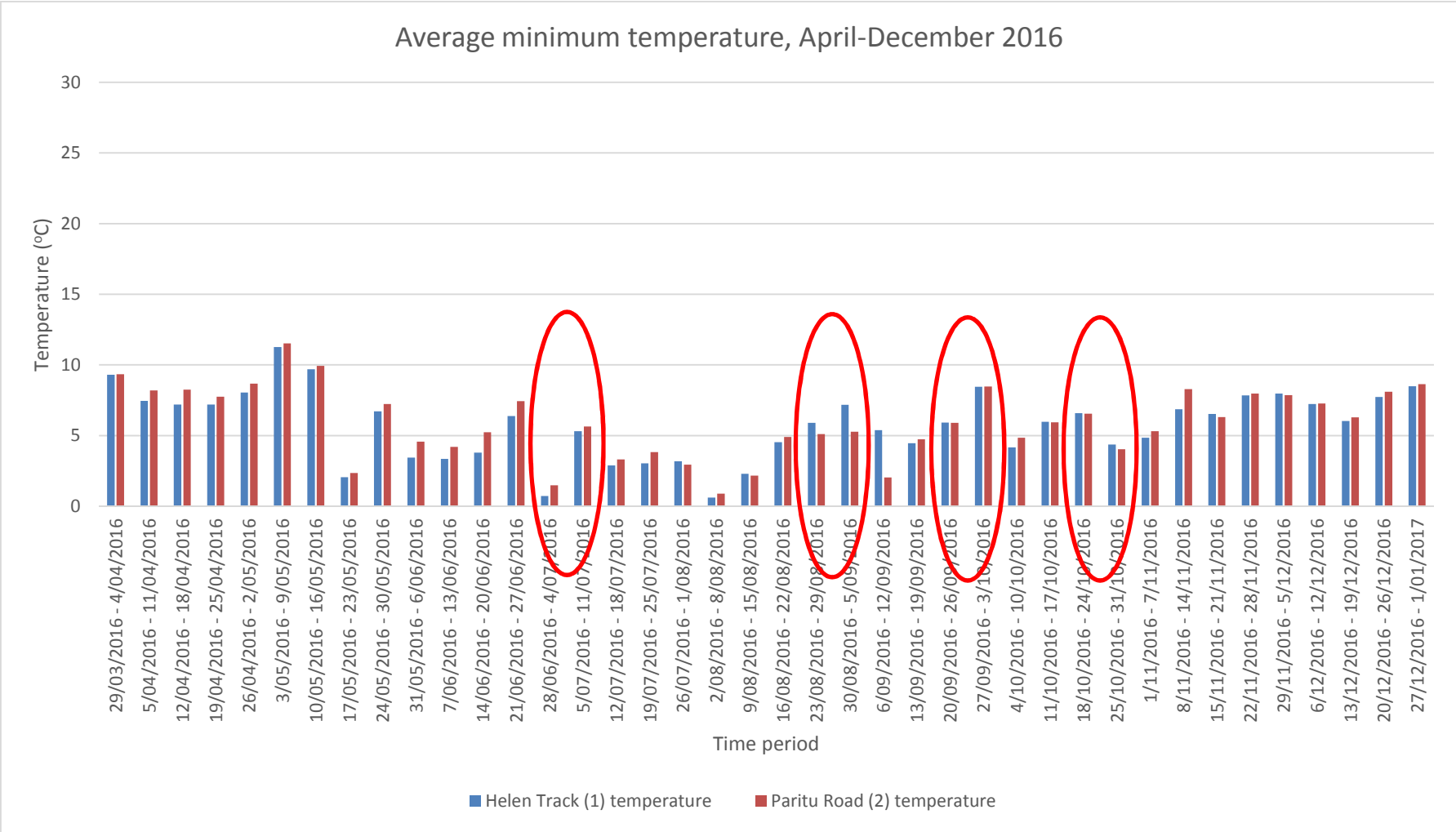

Figure 14. Average weekly minimum temperature (means of daily minimums) during the second season at Site1 (Helen Track) and Site 2 (Paritu Road). The red circles indicate the average minimum temperatures that occurred during the two-week exposure intervals in which infection was detected on exchange plants at nearby subsites (Table 4).

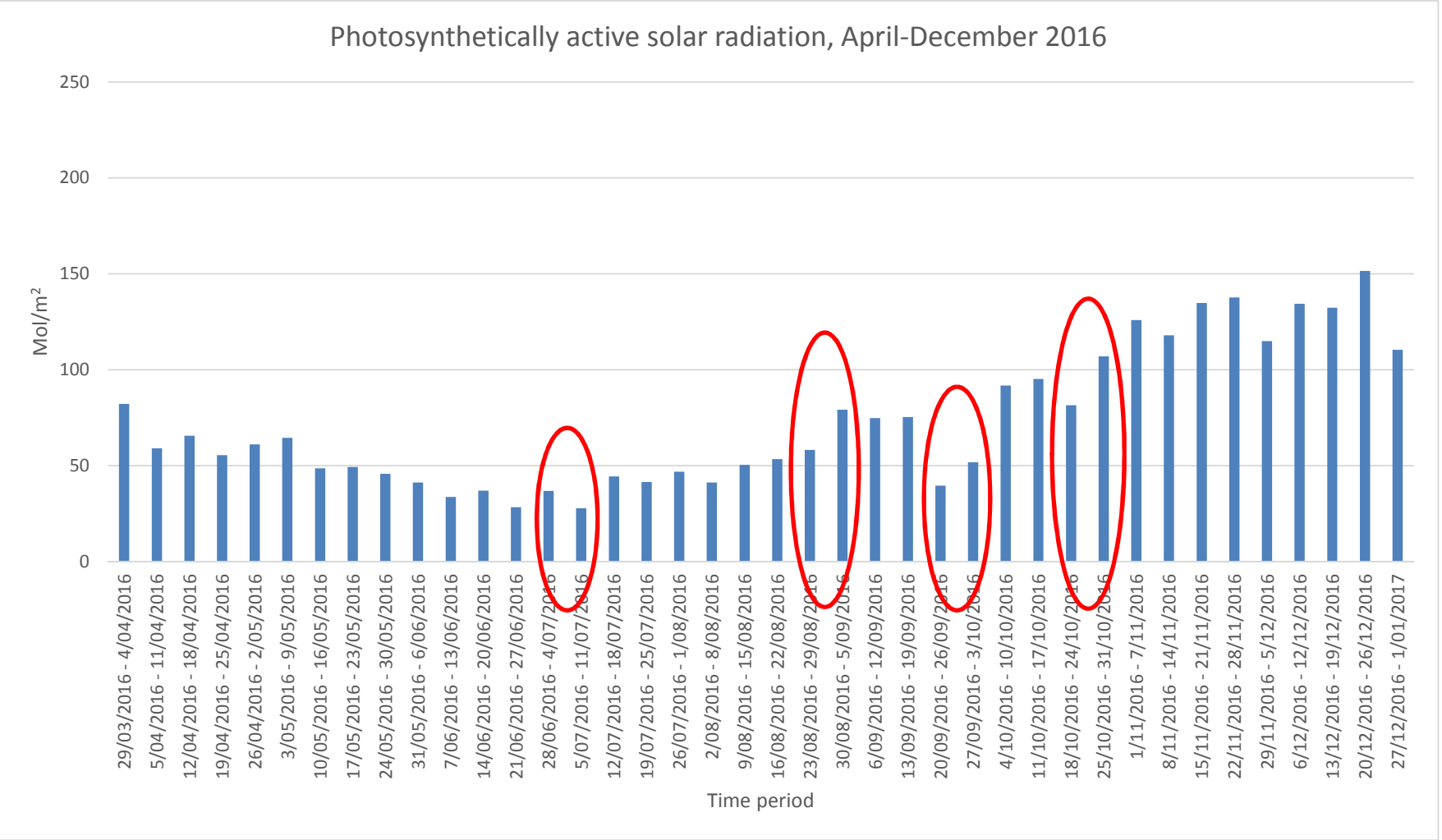

Figure 15. Total weekly, under-canopy, photosynthetically active solar radiation during the second season at Site 2 (Paritu Road; not recorded at Site 1). The red circles indicate the quantities of solar radiation that occurred during the two-week exposure intervals in which infection was detected on exchange plants at nearby subsites (Table 4).

4. Discussion

4.1. Introduction – the importance of knowing the pathogen life cycle

A good knowledge of the seasonal development of a pathogen, and the way it is affected by the environment, is essential to the efficient management of forest diseases. Only with this information is it possible to treat at optimum times and frequencies for cost effective control. However, understanding the life cycle of *P. pluvialis* has proved difficult because of the uneven behaviour of red needle cast year-to-year and our inability to predict where and when it will appear. Despite this, and although limited in scope, the latest study has added significantly to the broad picture, bringing it more clearly into focus. Previous work has shown indirectly that infective propagules (presumed to be swimming zoospores and possibly also detached sporangia) are available for infecting foliage through much of the year (Williams et al. 2016). The aim of the current series of studies using exchange plants was to resolve the question of when these propagules actually infect the foliage. For maximum efficiency one would expect a pathogen to release propagules only when conditions are suitable for infection to occur. However, for some species, production and release of reproductive bodies may be prolonged and extravagant, increasing the likelihood of at least some infection even when conditions may be only marginal for it to occur, especially if a minimum number is required for success. Additionally, when both host and pathogen are exported to an exotic environment their phases of development may no longer be precisely synchronised. In New Zealand, fruit bodies of the Swiss needle cast pathogen, *Phaeocryptopus gaeumannii* (Rohde) Petrak, begin producing ascospores in quantity at least a month before susceptible new foliage appears during the November flush of its Douglas fir host (*Pseudotsuga menziesii* (Mirb.) Franco).

4.2. What we know

Table 7 presents a summary of what we currently know about the life cycle of *P. pluvialis* from the previous and present studies in the North Island (a similar table for *P. kernoviae* is included as App. B). It demonstrates that in certain high disease severity periods, inoculum may be available through more than half of the year (e.g. at Wharerata in 2012). It also shows that in other years available inoculum may be negligible to non-existent (e.g. at Kapenga in 2013 and 2014[7]). Within the periods with available inoculum, infection has been found to occur at least during July, August, September and October (Table 7). It cannot be assumed that infection is restricted to these months, because of the limited number of exchange plants (the results in the two successful studies are qualitative and indicative rather than quantitative, and not amenable to statistical analyses)[8]. It is clear that some infection takes place before July (infection plainly occurred in the diseased stand trees prior to the setting up of the two subsites at the end of May), and one cannot rule out diminishing levels of infection after October, which might have been detected with larger numbers of field-exposed plants. Similarly, the negligible detection of available inoculum may be due to the small number of needle-baited traps, though it is still puzzling why there were only two positive identifications, at different subsites on the same date (and why laboratory controls were not always effective).

[7] These inoculum free years (2013, 2014) coincide with the periods in which the disappointing plant exchange studies were run, also at Kapenga (Hood et al. 2014a, Hood and Uaea 2015).

[8] As previously, many plants were set up at the start of the present study (balancing quantities against costs of grafting and maintenance), but numbers deployed in the field were constrained by factors such as limited vehicle space during transport to Wharerata and the need to deploy individual plants only once during much of each year. The establishment of the subsites further spread the available numbers of plants even thinner. Occasional unrelated foliage browning in both seasons rendered a number of plants unusable.

Table 7: Summary of current knowledge of *P. pluvialis* life cycle based on present and previous studies in two North Island regions (Kapenga, near Rotorua, and Wharerata, near Gisborne). Approximate periods of generally fortnightly field exposure are indicated by shading. Occurrence of at least some infection and available inoculum over these periods is shown by darker shading.

Month	Infection occurrence (indicator plants)		Inoculum availability (needle baited spore traps)						
	Wharerata (this study)	Kapenga (Hood et al. (2013a)	Wharerata (this study)	Kapenga (Williams et al. (2016)			Wharerata (Williams et al. (2016)		
	2016	2012[1,2]	2016	2012	2013	2014	2012	2013	2014
January									
February									
March									
April									
May									
June									
July									
August									
September									
October									
November									
December									

[1]Monthly exposure periods (approximately).

[2]Needle symptoms also seen on plants exposed in June; likely, but not confirmed as *P. pluvialis*.

4.3. Relationship with the environment – completing the disease triangle

An important aim of this study was to identify environmental factors that may promote infection. It had previously been determined (Williams et al. 2016) that available inoculum is more common during the cooler months of the year. Table 7 indicates that, with exceptions, inoculum does not generally become available for infection until autumn and tends to disappear by December, when temperatures rise and as trees lose their diseased foliage, the source of the inoculum. It was also found that inoculum was detected more frequently following periods of rainfall. Results from the present study support these trends and show that in general infection took place during the cooler part of the year[9] after a period of rainfall (small plant numbers may explain the failure to detect infection following one period of rainfall early in August 2016). Free water will enable dispersal and germination of spores and facilitate entry into the needle from the surface. Number of rain days and minimum temperature were also examined in order to allow comparison with previous work which used these units (Williams et al. 2016). Inspection shows that outcomes were similar. In the previous work, available inoculum of *P. pluvialis* was detected at Wharerata at average minimum temperatures around 7 °C after ca. 6-7 days of rainfall in a 14-day period (but not at 8.5-9 °C or after 5 rain days; Fig. 9, in Williams et al. 2016). These findings were based on calculated weather data for a virtual climate station within a distance of 5 km, supplied by NIWA. The present study indicated that 3 rain days per week (i.e. 6 days in a fortnight) are adequate for infection to occur at average minimum temperatures ≤ 7 °C[10].

Inoculum availability and infection also occur during the period of seasonally lower solar radiation, and it has been suggested that reduced insolation is a contributory factor to disease development. It is known that ultraviolet light, which will be less intense during autumn and winter, may be harmful to zoospores (McKee 1969; though, conversely, may also promote production of sporangia, Erwin and Ribeiro 1996). Mizubuti et al. (2000) and Aylor et al. (2001) found that solar irradiation significantly reduced the viability of sporangia of *Phytophthora infestans* (Mont.) De Bary. In this study only photosynthetic light was measured, beneath the canopy, but it seems a reasonable assumption that when there is more visible light there will be comparably greater ultraviolet radiation[11]. Because seasonal solar radiation paralleled that of temperature during the trial period, it is not strictly possible to distinguish which may be the principal variable. It is possible that both are relevant, with lower solar radiation during cooler months allowing propagules produced at lower temperatures to survive and infect during this period. Temperature, at least, is known to be a major factor influencing the behaviour of plant pathogens. With respect to *P. pluvialis*, it will require laboratory studies on the effect of ultraviolet light on sporangia and zoospores, and a controlled inoculation study that includes both parameters, to clarify this question.

[9] Footnote repeated from Hood et al. (2013a); cf. Table 7: "*P. pluvialis* may behave similarly in Oregon. Everett Hansen, Oregon State University (email of 6 November, 2012, to Margaret Dick, regarding *P. pluvialis*): 'It is quite striking—almost all of our old records come from rain traps in Jan, Feb, and March. Seems to contrast with [*P.*] *ramorum* that sporulates whenever it rains'. These months correspond approximately to July, August and September in New Zealand. November-March are the coldest months at locations in Oregon". Average monthly rainfall at Brookings, Oregon, between November and March ranges between 240 and 369mm (at other times between 11 and 165mm). cf. Hansen et al. (2015).

[10] There is a need to complement and verify this information by means of inoculation studies in controlled environment cabinets, and to learn the minimum quantity of inoculum needed. See Footnote 25.

[11] The summer radiation peak was lower in the second year of this study (App. A (f)), possibly because of an increased quantity of foliage on the crowns of trees above the recording station. As noted elsewhere, there was no disease and associated defoliation at the sites where the stations were sited.

4.4. Epidemic development

The results from this study imply that the high red needle cast severity observed in certain years, results from an epidemic situation that may develop due to more than one cycle of infection (a polycyclic epidemic[12]). Sporangia are produced within the same season following initial infection and a repetitive production of infectious propagules is suggested by the sustained detection of inoculum in spore traps during high severity years (Table 7). During such years, red needle cast symptoms on crowns of affected trees increase to a maximum during winter and spring, when most records are reported, before declining (Fig. 3 in Kimberley et al. 2016). The intensity of an epidemic depends on the rate that infection continues repeatedly to occur and on the amount of initial inoculum, both of which remain unknown for red needle cast. The rate of infection will depend on the length of the pre-reproduction period (the interval between infection and release of zoospores) which will be influenced by weather variables[13,14]. This period may vary seasonally and during part of the year could be quite short[15]. Sporangia have been found to occur on detached needles within 11 days of inoculation (J.F. Gardner, unpublished data), but more authentic information from similar work using living, still-attached needles does not yet appear to be available. We are also unaware of the nature of the initial inoculum and the way *P*. *pluvialis* survives during the interval between high severity disease years. The observation that the disease seems to recur periodically in the same stands (and often not in other trees nearby) might suggest that the pathogen is present in some form in these localities during lull periods, ready to produce infectious propagules when conditions again become suitable[16]. The role of oospores as resistant resting spores needs to be further investigated, though some initial study has been done (Williams, Dick, reported in Hood et al., 2014b). However, *P*. *pluvialis* may survive between years in some other form[17].

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[12] By contrast, *P*. *gaeumannii*, for instance, is monocyclic, with only one reproductive cycle each year.

[13] The rate of infection is also likely to depend on the degree of host genetic susceptibility (work of Nari Williams) and on the length of survival of available infective propagules (work of Peter Scott). At 10 °C, following a rapid initial discharge at relative humidities between 33 and 97%, some detached sporangia survived and continued to release zoospores for up to 6 days (Scott 2016).

[14] In this study, sporangia were only looked for and identified on exchange plants several weeks after they were returned to a different environment in the nursery, so it is unclear when they formed (though even in this situation they were still produced following infection within the same season), but those on the positive controls definitely appeared while these plants were still in place in the forest. Hence, the pre-reproduction period lies at least within the rather broad interval between when these positive control plants were placed at the new subsites (31 May) and when sporangia were first looked for and noticed (15 November). However, the pre-reproduction periods are probably much shorter, potentially with multiple cycles in a season. Study records indicate that sporangia were often empty, at least on returned exchange plants (Fig. 7), indicating that zoospores had been released. Variability in the length of the pre-reproduction period may lead to some overlapping (or "blurring") of cycle phases.

[15] It is possible that periods between infection and sporulation may be episodic, with a tendency to synchronise with intervals between occurrences of rainfall. It is evident from the earlier work (Williams et al. 2016), supported by this study, that there is an association between incidence of rain, on the one hand, and availability of inoculum and occurrence of infection, on the other. Therefore, infected needles may produce sporangia, release zoospores and initiate new infections only when rainfall or other forms of surface wetting take place (c.f. Fig. 10). Hence rate of infection (r) would be greater with higher rainfall frequency (rainfall at this time of year is plentiful in both New Zealand and western Oregon, refer Footnote 9). Does an infected needle produce successive crops of sporangia during recurrent periods of rain before it is shed? A careful inoculation study should clarify these points.

[16] This is apart from the additional likelihood that in some localities or zones e.g. at cooler elevations prone to fog or dew, or in moist valleys, conditions are more conducive to the disease (as also occurs with *D*. *septosporum*).

[17] Possibilities may include infection of living rootlets in the soil (work of Peter Scott, Rebecca McDougal, pers, comms.) or perhaps by symptomless infection of green needles (or of foliage or shoots of another unknown host plant species nearby?). *Phytophthora kernoviae* was recovered from soil at a central North Island site between April and November by baiting for zoospores (Gardner et al. 2015). But how, then, does inoculum reach tree foliage? Ascospores of *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter are forcibly discharged from colonised litter but conidia of *D*. *septosporum* are only dispersed from infected, still attached needles (P.D. Gadgil, *pers*. *comm*.).

4.5. Local dispersal

Although not proven statistically, it was quite noticeable that infection took place almost exclusively at the subsites close to (but not immediately beneath) diseased stands supplying the infectious propagules (especially at Subsite 1, where stand disease was greater)[18]. This result implies that the majority of inoculum does not travel far in drip or rain splash during wet weather[19]. This presumably helps explain why the disease often occurs contiguously in groups of adjacent trees, at times with only the inner-facing sides of crowns affected on those at group margins. It is also common to find young diseased naturally regenerating seedlings and saplings beneath heavily infested stands (Fig. 16). Time will tell whether or not the disease is slowly spreading to new localities within its already widespread distribution. This implication of mainly limited spread does not preclude a small amount of more widespread wind dispersal in aqueous aerosols. Is there also a possibility that some disease may have been moved around on infected nursery stock?

4.6. Pathogen life cycle and disease management

Understanding the pathogen cycle is highly relevant to disease control. For instance comprehensive work with dothistroma needle blight in the 1960s and 1970s (Gadgil 1967, 1970, 1974, 1977; Gilmour and Noorderhaven 1971; Gadgil and Holden 1976; Gilmour 1981) showed conclusively that spores (conidia) produced in sufficient numbers from infected, still attached needles disperse in rain splash to new needles which they infect. This occurs, if the weather is wet, once temperatures have warmed sufficiently during November or December. Needles infected at this time eventually produce new fruitbodies and release further spores around February, if rain occurs, leading to a second cycle of infection. It was found that spraying with a copper fungicide at these critical times controls the disease by stopping fruitbody production and spore release (Bulman et al. 2004). To a lesser extent, the fungicide also kills and prevents spore germination thus protecting needles from entry and invasion. Spread of *D*. *septosporum* spores locally between stands has been shown to be limited as also appears true for *P*. *pluvialis*, as indicated above. However, by contrast, *P*. *pluvialis* appears to prefer a cooler time of year for its development.

Results so far therefore suggest that with *P*. *pluvialis*, a fungicidal spray treatment may be effective if applied during autumn, as temperatures, are beginning to cool, with possibly a requirement for a second spray later in the season (Table 7). The appearance of propagules during March at two locations in 2012 suggests that this is when infection commences and hence when a first treatment spray should be applied to prevent sporulation. The detection of propagules in traps during January, 2014 (Table 7), is initially disconcerting, but this is a continuation of positive trapping from late 2013 and propagules may not have been produced had the disease been controlled in that year. However, our knowledge is limited and it is clear that more needs to be learned about the nature of the initial inoculum, when it first infects new foliage and how frequently cycles of infection occur, before more precise management recommendations can be made[20]. An understanding of

[18] Only on two needles on one permanently placed, non-exchange plant at an original site, several hundred metres from any diseased trees, was there any indication of possible infection by *P*. *pluvialis*. It is perhaps serendipitous, after all, that red needle cast was only patchy during the second season of this study. Had the disease been more widespread, as in some earlier years, this limited dispersal of infective inoculum may not have been apparent. *Dothistroma septosporum* behaves similarly.

[19] This further suggests that dispersal is mainly by swimming zoospores. By contrast, *P*. *infestans* covers greater distances by means of air-borne, dehiscent sporangia (e.g. Aylor et al. 2001).

[20] The theoretical model for a polycyclic epidemic is $x=x_0e^{rt}$, where x_0 is the initial disease incidence (proportional to initial inoculum; expressed as a value between 0 and 1), x and r are the proportion of disease and the apparent rate of infection, respectively, at time t, and e is the base of the natural logarithm ("apparent infection", based on visible symptoms, trails actual infection). The model changes as the sigmoid curve flattens towards the climax of the epidemic (r decreases towards zero and x plateaus at its maximum). Hypothetically, to control a polycyclic epidemic one must reduce ***both*** x_0 (initial inoculum) and r (rate of infection). It appears that copper fungicides may do both, with *P*. *pluvialis*.

which factors govern the periodicity in disease severity in successive years is also needed. Nevertheless, it is encouraging that the copper fungicides used economically for dothistroma control also appear to be effective against the red needle cast pathogen, and that the effect appears to be long-lasting (Rolando et al. 2013, 2017; Gous et al. 2015)[21].

Fig. 16. Young, self-sown *P*. *radiata* with red needle cast beneath diseased plantation trees. Above: Rotorua to Tauranga direct route, 8 August 2016. Below: near Kinleith, 6 October 2015.

[21] It is interesting that one of the formulations that showed effectiveness in a study testing the ability of operational anti-sapstain products to kill oospores of both *P*. *pluvialis* and *P*. *kernoviae* placed on a bark surface contains a copper compound as one of its ingredients (Hood et al. 2013b).

4.7. Where to next?

Although a picture has emerged of the way red needle cast develops, suggesting broadly when a treatment or treatments should be applied, there is clearly a lot more to be learned before a recommendation can be made with confidence. The achievements to date are largely indicative and require stronger confirmation. However, work so far has demonstrated techniques that may be used successfully, given the right circumstances. Field research is hampered by the irregular occurrence of outbreaks of red needle cast both regionally and year to year. This aspect can be overcome in part by expanding the studies that will provide the key answers. It is a question of priorities and resources, and how severely forest managers consider the disease is impacting on their plantations. More plants and spore traps at a greater number of sites each year, possibly with some "in kind" assistance from forest management, will increase the chance of successfully running a study in a severely diseased stand through one or more complete years, accumulating the necessary data. There is also other work that needs to be accomplished, in both the field and laboratory, that is not dependent on the irregular nature of the disease. The extensive information derived from the thoroughly conducted studies during the early dothistroma research years provides an excellent model. The following suggestions are submitted as to the manner in which it is believed research into disease development should be directed in order to solve the essential questions as quickly but as carefully and rigorously as possible.

- Undertake a more comprehensive, confirmative, **plant exchange study to determine infection period (and hence when and how often to spray fungicide)**, upgraded and quantifiable (able to be statistically analysed) as discussed above, including inoculum traps and with on-site meteorological stations. It may be found acceptable to facilitate the work by using "normal" *P*. *radiata* seedlots, which possibly have sufficient general susceptibility to *P*. *pluvialis,* rather than by preparing grafted cutting stock from trees known to be susceptible[22]. Use of grafts is more costly and takes time before ready.

- Conduct a **complementary timing trial with potted plants using a fungicide** found to be effective. Aim: also **to determine when and how often to treat**.

- Continue with **longer term monitoring** of red needle cast, to collect matching data sets of disease severity and weather variables. Aim: to **see if able to predict disease severity occurrences and therefore in which years to apply treatment**; to understand the environmental variables that affect rate of infection (r) in epidemic years.

- But disease outbreaks may depend on other factors besides weather[23] e.g. the degree of inoculum at the start of an epidemic (x_0). **Investigate the nature of the initiating inoculum** (soil, symptomless attached needles?). Conduct **a study with individual young stand trees to follow a potential epidemic from first infections**.

[22] This assumes that young *P*. *radiata* seedlings are as susceptible as older material. Seedlings have succumbed as readily as other stock ("mother plants") during high disease periods at the Arborgen Tokoroa nursery (Mark Ryan, pers. comm.), but material less than one year old may not be as vulnerable (Rebecca Ganley and others, unpublished inoculation data).

[23] In this study, weather variables in the first season appeared as suitable as in the second (App. A), yet there was no stand disease, available inoculum or infection (but is the higher rainfall in November 2015 and January 2016, than in the previous year, relevant? App. A(g); cf. P. 17 in Hood and Uaea 2015).

- Undertake an **inoculation study in controlled environment cabinets** to define parameters (temperature, leaf wetness etc.) for infection to occur[24]. This will support the field studies and provide information unaffected by vagaries of the disease that they are exposed to. However, it will be necessary to use reliable inoculum that is preferably quantified. Ideally, separate studies will determine aspects such as the pre-reproduction period under different conditions, the minimum inoculum concentration threshold to effect infection[25], the effects of light and ultraviolet radiation etc.
- Continue with the **environmental tolerance studies** (Peter Scott) to determine propagule survival under different conditions and how this effects the rate of infection.

5. Conclusions

- Using susceptible exchange plants it was determined that infection by *P. pluvialis* occurred at intervals, at least between 29 June and 18 October, 2016, at Wharerata Forest.
- Floating needle-baited traps demonstrated the availability of inoculum at least on 18 October, 2016.
- Infection on exchange plants generally occurred after a period of rainfall (at least 6 days of rain >2mm, or ≥ 150mm total rain, in a 14 day period) at average minimum temperatures ≤ 7 °C (or average temperatures ≤ 14 °C). Infection also occurred when the level of photosynthetically active solar radiation to which plants were exposed beneath the stand canopy was ≤ 100 mol/m^2/week.
- Infection occurred (with one exception) only on plants within ca. 20 m of a diseased stand, and not on those several hundred meters distant, indicating limited dispersal of inoculum.
- The appearance of sporangia on infected needles on exchange plants implies a polycyclic epidemic scenario for red needle cast. This needs to be verified.
- From this and previous research (Nari Williams) it appears that in high severity years, inoculum is first produced, leading to initial infection, from early autumn. Periods of rainfall promote further cycles of infection leading to epidemic disease development and greater symptom expression. Climax is reached during late winter and spring, followed by gradual defoliation over summer resulting in green but thin-crowned trees. However, more work is needed to confirm this understanding.
- If this interpretation is correct, it suggests that a control treatment should be applied in early autumn, to minimise inoculum at the start of a potential epidemic, followed if necessary by another several months later, to maintain a low rate of infection.
- There is a need to understand the nature of the initial inoculum and how it behaves, as this may also influence disease prediction and effective treatment timing.

[24] The decline of a red needle cast outbreak coincides with the warmer temperatures during spring and summer, which may be unfavourable to the pathogen, and hence the cause of the reduced rate of infection (r). But, alternatively, it may be the shedding of diseased foliage, thus removing the sporangia-producing infected needles from the system, that is responsible for the end of the epidemic; or both explanations may apply. It is evident from Table 7 that zoospore may sometimes be released in January, but does this lead to infection at this time? The range of temperatures over which infection can occur will be resolved by controlled inoculation studies.

[25] Some inoculation studies investigating ranges of temperature and zoospore concentration for successful infection have been undertaken (Rebecca Ganley and others, unpublished data).

6. Acknowledgements and contributions

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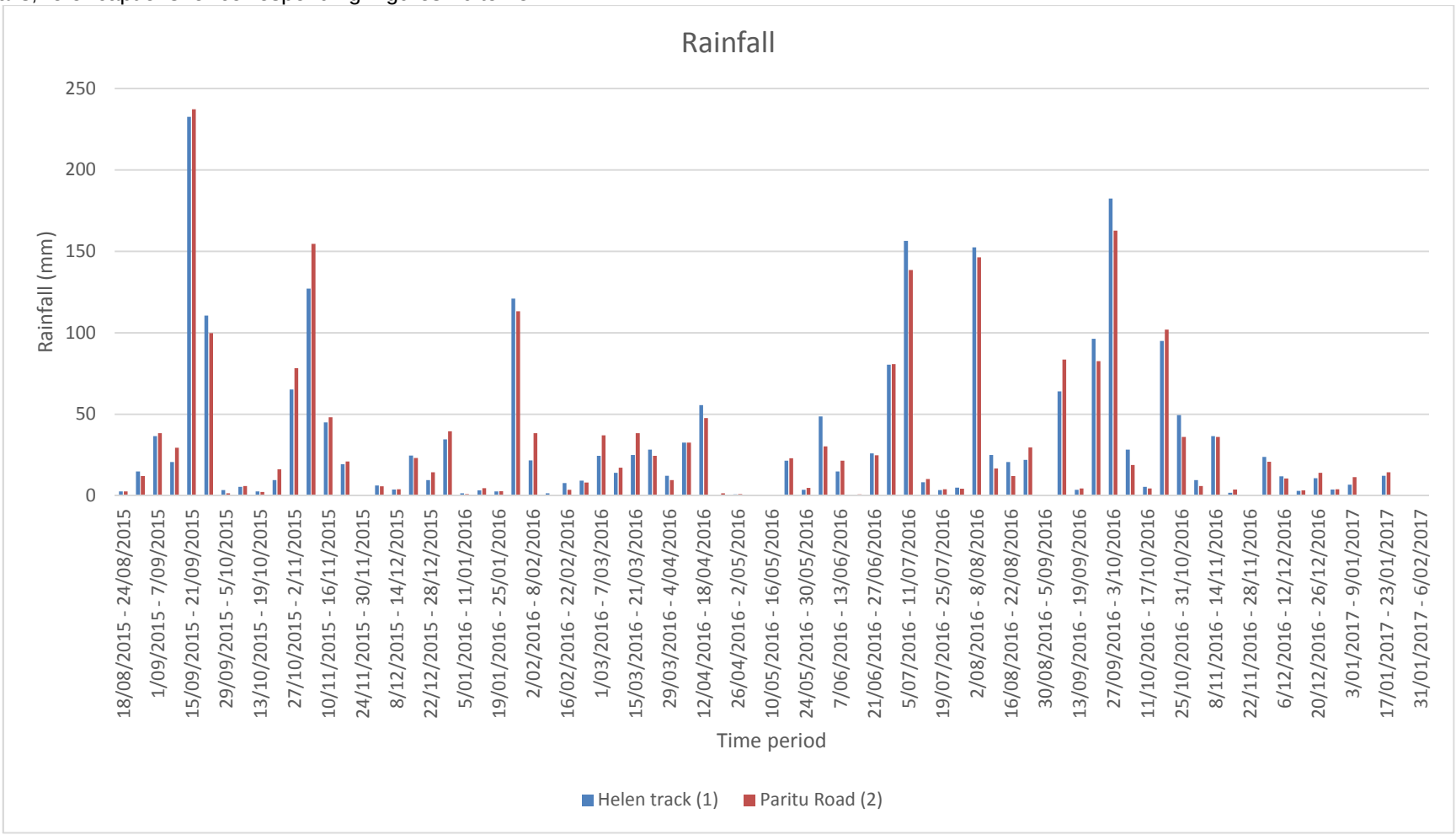
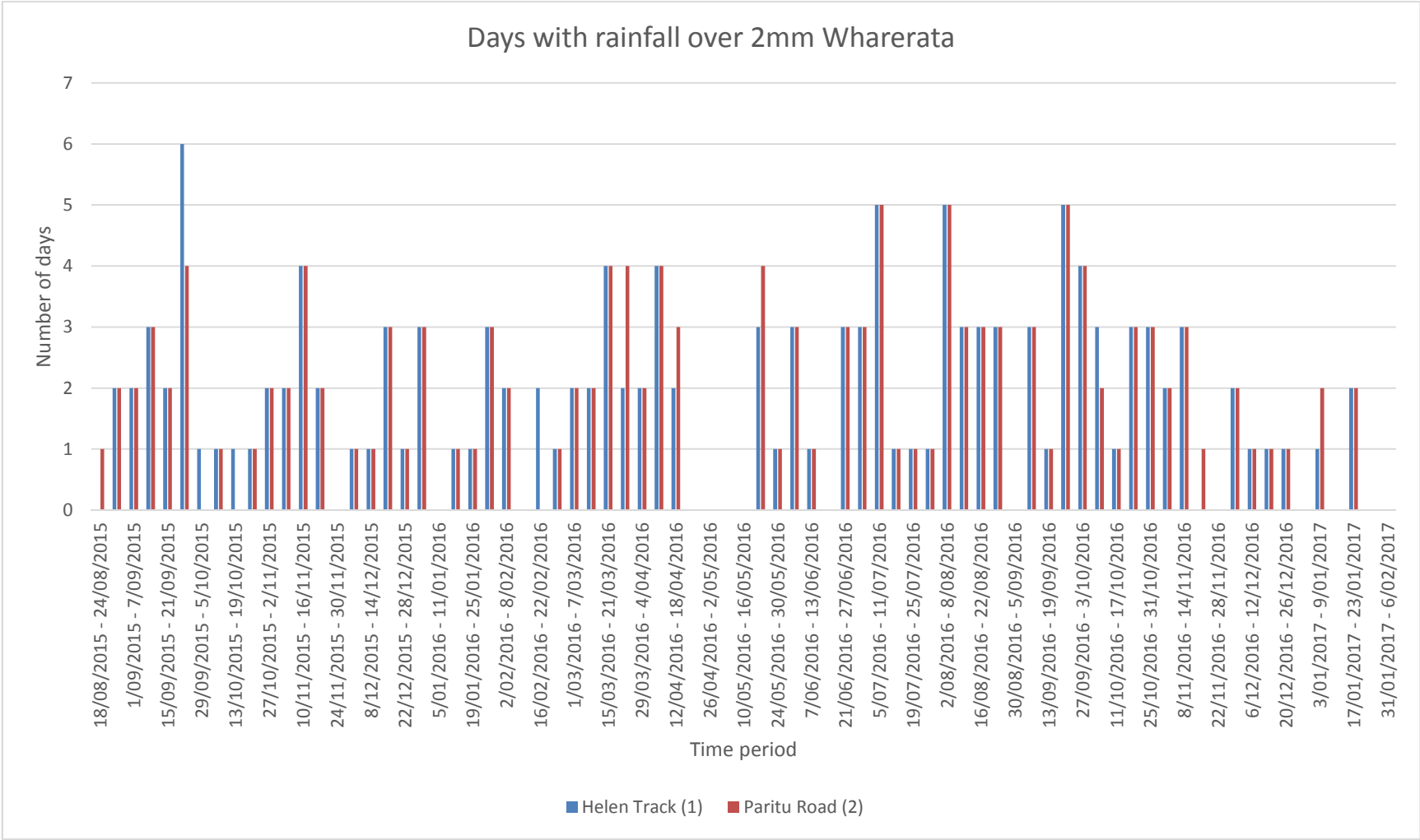
Appendix A

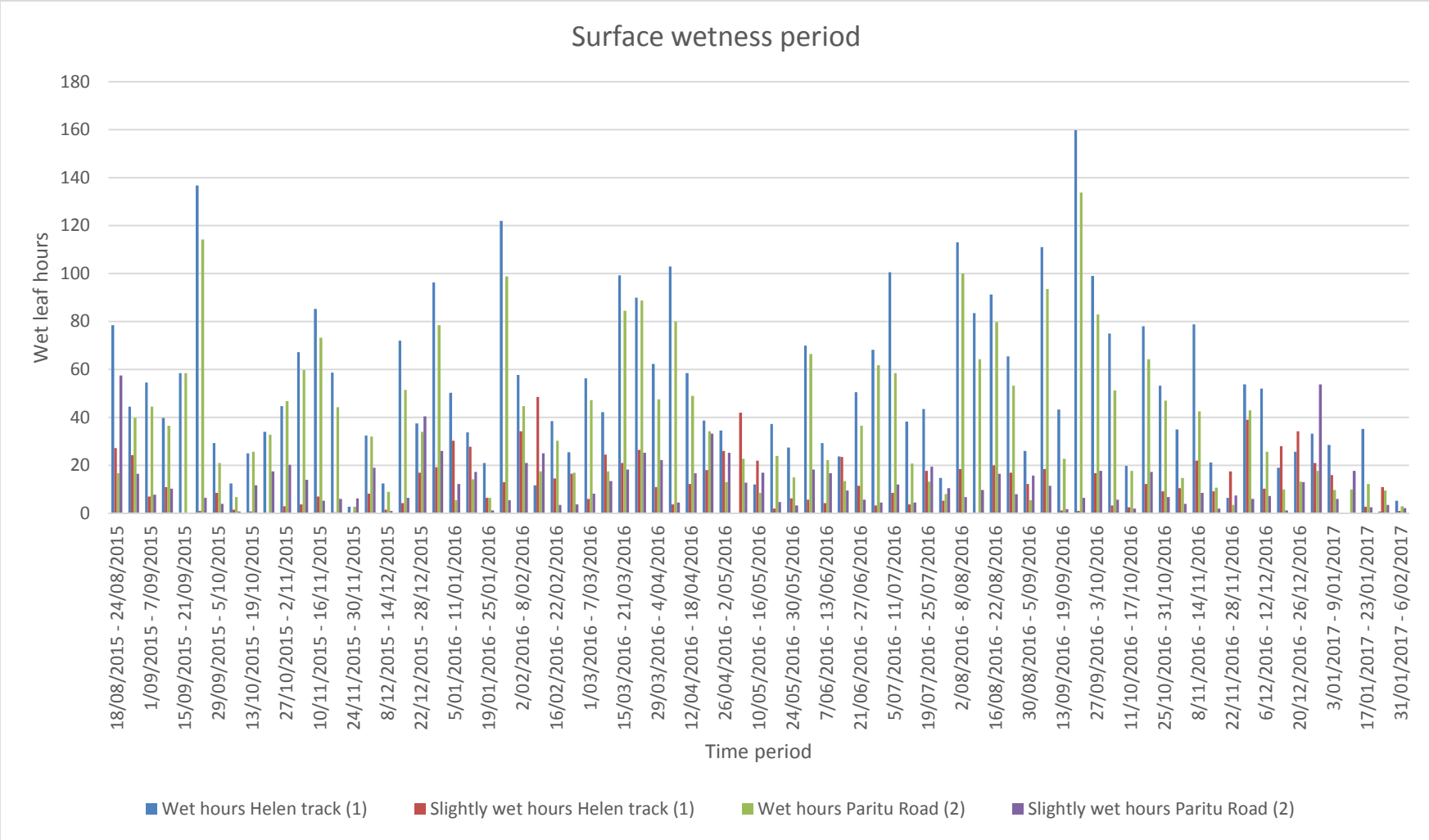
Graphical summaries of weather variables recorded at the meteorological stations at Site 1 (Helen Track) and Site 2 (Paritu Road) throughout the full trial period. For details, refer captions for corresponding Figures 10 to 15.



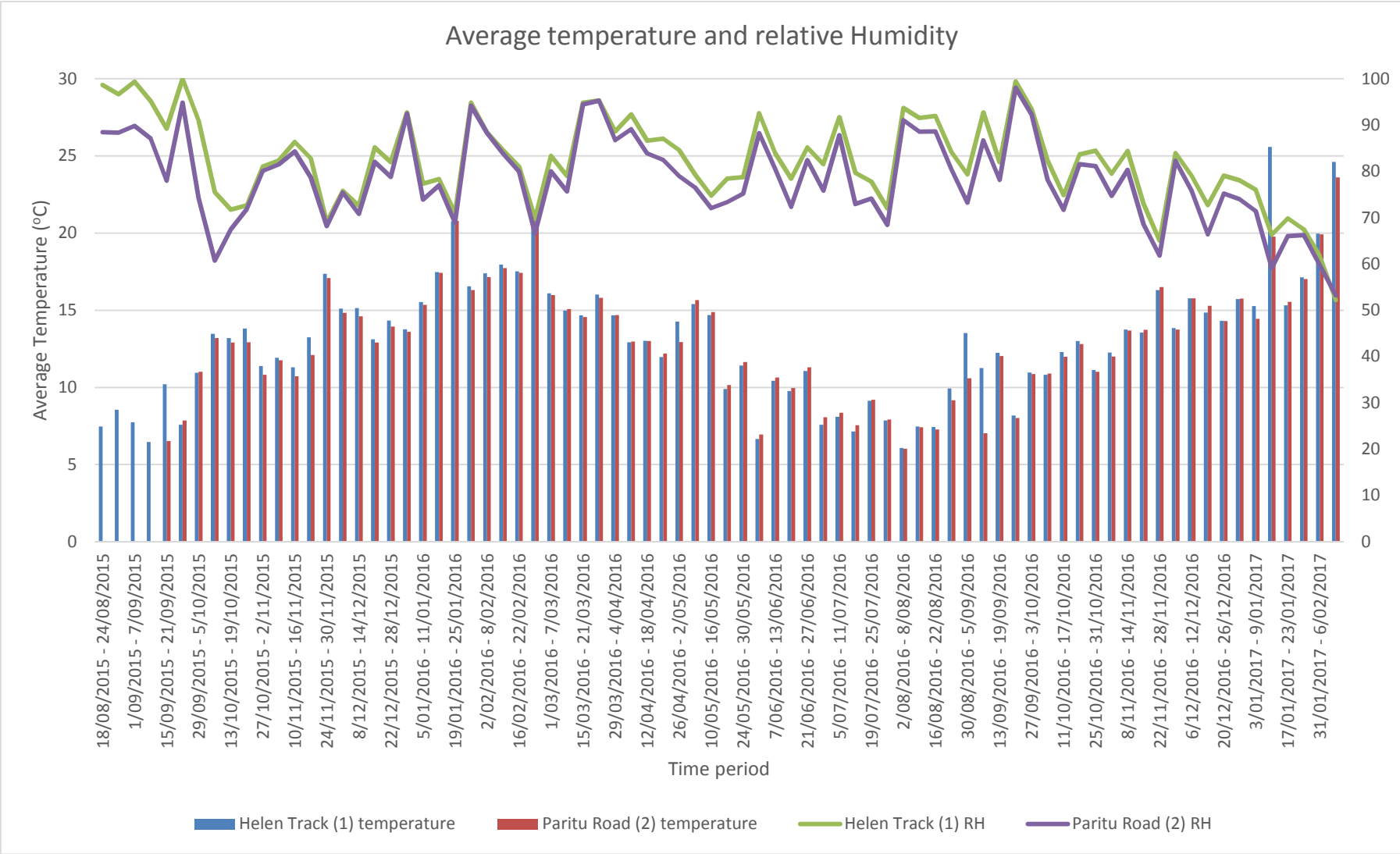
(a) Total weekly rainfall (refer Fig. 10).



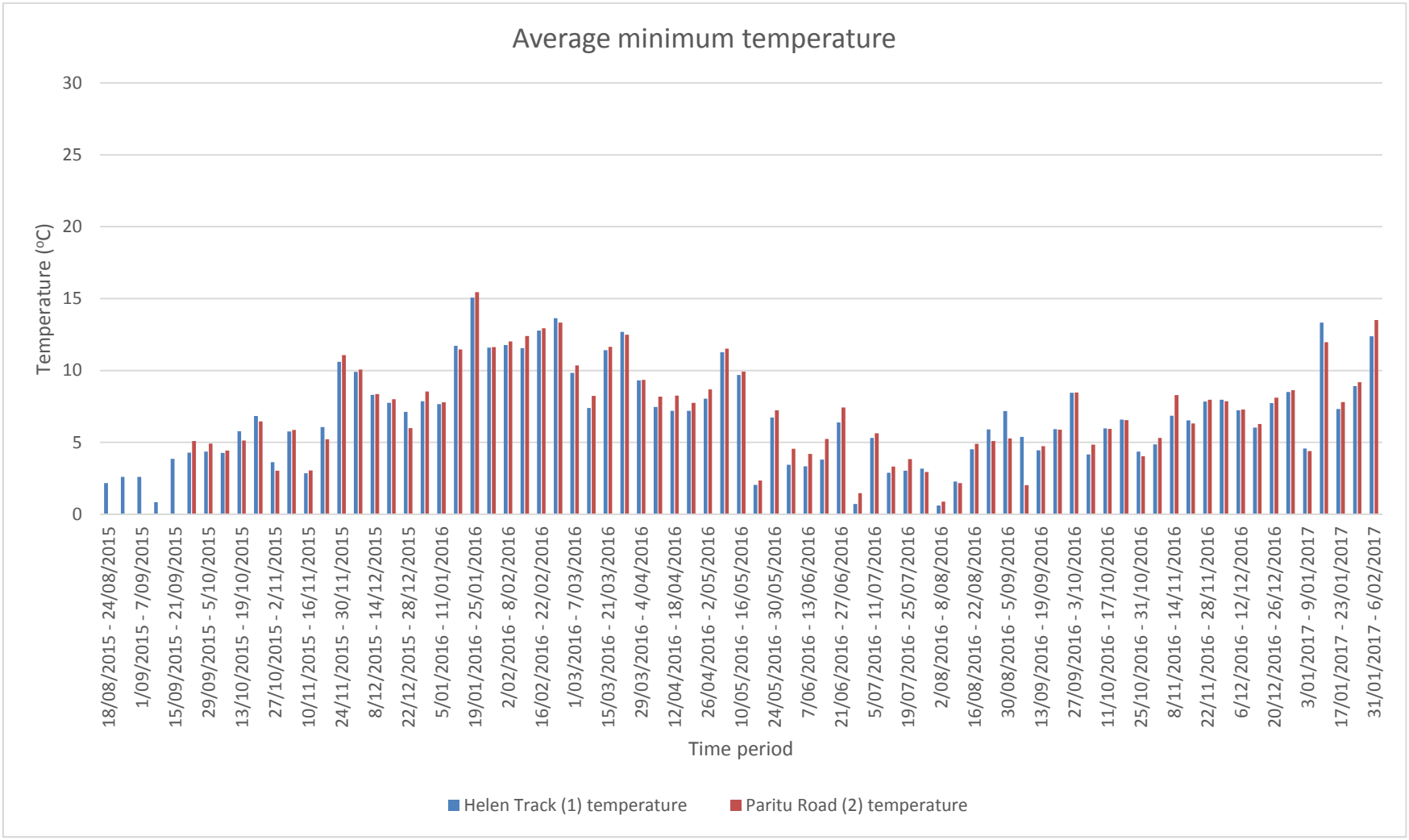
(b) Number of days per week with rainfall greater than 2 mm (refer Fig. 11).



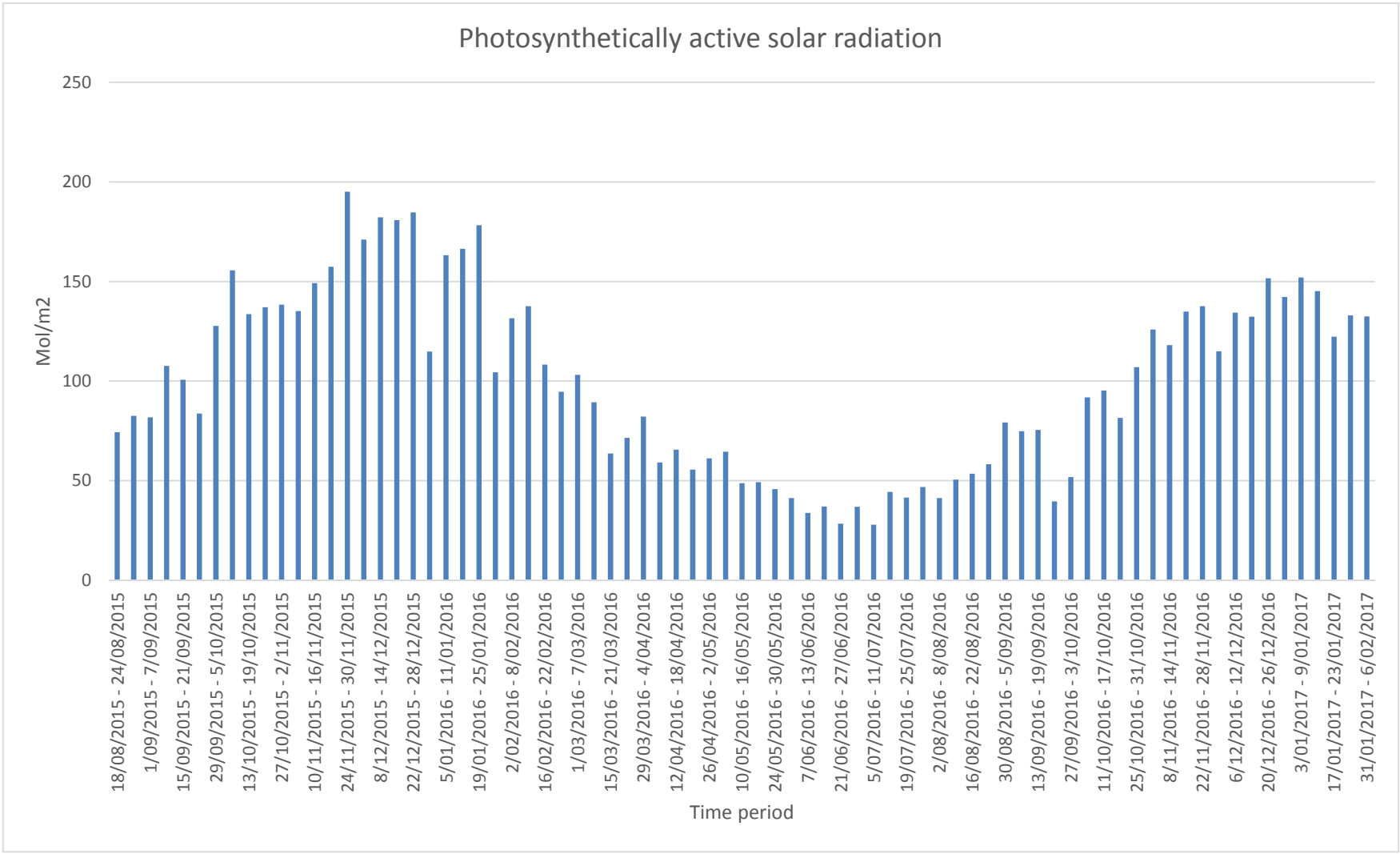
(c) Weekly surface wetness period (refer Fig. 12).



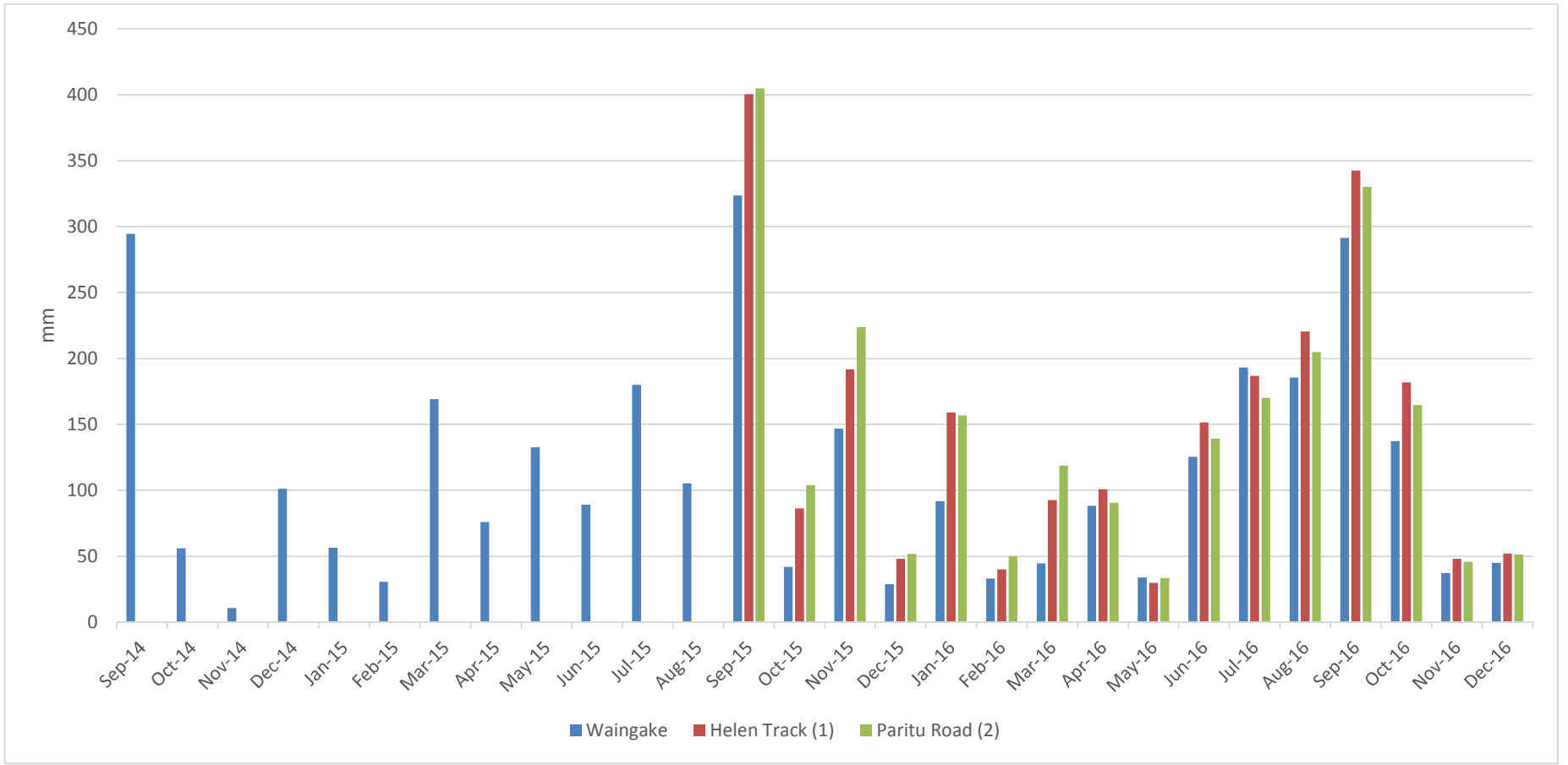
(d) Average weekly temperature and relative humidity (refer Fig. 13).



(e) Average weekly minimum temperature (refer Fig. 14).



(f) Total weekly, under-canopy, photosynthetically active solar radiation at Site 2 (Paritu Road; refer Fig. 15).



(g) Monthly rainfall, 2015-16, at Waingake, Site1 (Helen Track) and Site 2 (Paritu Road). Waingake NIWA meteorological station (177.7915°E/38.7966°S; elevation 140m a.s.l.) is situated 35 km to the north of Wharerata Forest (refer Footnote 23).

Appendix B

Summary of current knowledge of *P*. *kernoviae* life cycle based on studies in two North Island regions (Kapenga, near Rotorua, and Wharerata, near Gisborne). Approximate periods of generally monthly (infection monitoring) or fortnightly (inoculum monitoring) field exposure are indicated by shading. Occurrence of at least some infection and available inoculum over these periods is shown by darker shading.

Month	Infection occurence (indicator plants)	Inoculum availability (needle spore traps)					
	Tikitere (Hood et al. (2013a)	Tikitere (Williams et al. (2016)			Wharerata (Williams et al. (2016)		
	2012[1]	2012	2013	2014	2012	2013	2014
January							
February							
March							
April							
May							
June							
July							
August							
September							
October							
November							
December							

[1]Needle symptoms also seen on plants exposed in April, May and June; likely but not confirmed as *P*. *kernoviae* (only species ever found at this site).