Neonectria ditissima spore release and availability in New Zealand apple orchards

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Abstract Conidia and ascospore release of *Neonectria ditissima*, the causal agent of European canker, was investigated using rainwater traps and Vaseline®-coated glass slides in the Tasman region, New Zealand. Trapping of spores was carried out from May 2013 to June 2015 in three separate apple orchard blocks planted with 'Scifresh'/Jazz™, 'Royal Gala' and 'Braeburn', respectively. Conidia and ascospores were both produced at any time of the year when rainfall occurred. The numbers of both conidia and ascospores trapped peaked in April–May, but were produced throughout the year. There was a trend for lower spore numbers with increasing number of non-rainy days prior to rainy event ≥2mm, while more spores were trapped under frequent rainfall conditions. There was a significant correlation between mean conidia trapped and total monthly rainfall in all three orchards, but ascospore numbers were significantly correlated with rainfall on glass slides only in the 'Braeburn' orchard.

Keywords *Neonectria ditissima*, sporodochia, conidia, 'Braeburn', 'Royal Gala', ascospores, European canker, 'Scifresh'/Jazz™, perithecia.

INTRODUCTION

European canker (EC), formerly referred to as Nectria canker or apple canker, is caused by the fungal pathogen *Neonectria ditissima* (formerly *Nectria galligena*). The pathogen has been reported from all regions of the world known for commercial production of apples and pears (CPC 2005; Beresford & Kim 2011). The fungus produces ascospores from perithecia and conidia from sporodochia that are both produced from cankers on infected wood. Both spore types are dispersed, and can cause significant infection, during prolonged periods of rainy weather (Wilson 1966; Xu & Butt 1996). Sporulation, spore dispersal and infection of *N. ditissima* have been observed to be favoured by rainfall (McCracken et al. 2003). Spores infect by entering host tissue through wounds. Infection has been associated with pruning, grafting, budding and broken branches or through non-callus scar tissue, petal scars, leaf scars, fruit picking scars or growth cracks (Swinburne 1971).

Some authors have reported regional differences in spore production and maturation. For example, in Tasmania, Australia, Ransom (1997) reported that EC perithecia did not reach maturity and no ascospores were released.

However, in the Nelson/Tasman and Waikato regions of New Zealand, matured ascospores produced from perithecia in older canker lesions has been observed (R.W.A. Scheper unpubl. data). In Germany, ascospores are particularly important and both conidia and ascospores are available all year round except for dry periods and freezing conditions in winter (Weber 2014). Knowledge of *N. ditissima* spore availability and the climate drivers thereof is required to optimise disease management practices. However, no reports exist on spore release patterns under New Zealand climatic conditions. The aim of this study was to determine the seasonal release and availability of *N. ditissima* spores. Understanding such factors will facilitate prediction of *N. ditissima* infection risks and improved EC management decisions.

MATERIALS AND METHODS

Spore trapping was carried out in three separate commercial orchards near Motueka in the Tasman region. The orchards were approximately 7-km apart. Orchard 1 was planted with Royal Gala, orchard 2 with 'Braeburn' and orchard 3 with 'Scifresh'. Two different types of trap designed to trap ascospores and/or conidia were setup in these orchards. The choice of the trapping device was based on the position of the canker. Spores from cankers on stem (vertical) were trapped using slides and cankers on horizontal branches were trapped using rain traps. European canker (EC) management practices in the 'Royal Gala' and 'Scifresh' orchard blocks involved fungicide applications and EC tree incidence as at the time of spore trapping was 100 and 70%, respectively. The 'Braeburn' orchard block had no fungicide applications and EC tree incidence was estimated to be 50%. Lesion sizes were much smaller in the 'Braeburn' orchard block than that of the 'Royal Gala' orchard block. In all the orchard blocks, cankers ranging from 50 to 100 mm in length with either sporodochia or perithecia visibly present were initially selected and labelled for spore trapping.

Rainwater traps in 'Scifresh' and 'Royal Gala' orchard blocks

To determine the availability of *N. ditissima* conidia during rainfall, rainwater traps were installed in the 'Royal Gala' and 'Scifresh' blocks. Each rainwater trap was constructed from 3.5-cm diameter PVC pipe (22-cm long) that was cut in half lengthwise to form a channel and mounted slightly off-horizontal, below a cankered branch. A plastic tube connected the lower end of the PVC channel to a 1-litre bottle where rainwater dripping from the cankered branch was collected for spore counts. Four traps were installed in each orchard and drip water was collected after rain events ≥2 mm/day from May 2013 to June 2015. The quantity of rainwater collected in each trap was measured, centrifuged, the spore pellet was re-suspended in 50 mL of tap water and spores characteristics of *N. ditissima* counted using a haemocytometer. Spore counts were expressed as numbers of ascospores or conidia per millilitre of water collected per trapping event.

Vaseline®-coated glass slide traps in 'Braeburn' and 'Royal Gala' orchard blocks

Vaseline® (white petroleum jelly)-coated glass microscope slides were used to determine the availability of *N. ditissima* spores during periods with and without rainfall. Six slides were fixed vertically 5–7 cm from the surface of trunk cankers in each of the 'Royal Gala' and 'Braeburn' orchard blocks. The 'Royal Gala' orchard block was the same orchard that had rainwater traps set up. The slides were held by fold-back clips tied with wire to the stem. Slides were changed weekly when there was no rainfall or whenever there was rainfall ≥2 mm/day. The 'Royal Gala' orchard block was monitored for spore availability from June 2013 to June 2014 and the 'Braeburn' orchard block from June 2014 to June 2015. Trapping was moved from the 'Royal Gala' block to the 'Braeburn' block in the second year because the 'Royal Gala' block had been scheduled for removal due to the high disease incidence.

In the 'Royal Gala' orchard, five additional slides were fixed 5–7 cm from the surface of trunk cankers and were collected and replaced on five occasions only while it was still raining or drizzling. Seven other slides were installed on wooden posts and placed randomly in the orchard away from canker lesions at a height of 1 m. These random slides were collected and replaced weekly. Spores characteristic of *N. ditissima* collected on the slides were counted under a compound microscope at ×200 magnification along three scans along the breadth of each slide. The total number of spores per mm^2 was determined for each exposure period.

Statistical analyses and weather data

The total monthly conidia and ascospores were log10 (x+1) transformed and means and standard errors calculated using Excel pivot tables. Mean monthly numbers of spores trapped from the various orchards were correlated against the mean monthly temperature and total monthly rainfall using Pearson correlations (calculated in GenStat version 17). Daily rainfall (mm), daily mean temperature (°C) and surface wetness for the Riwaka Plant & Food research station was about 3 km to the 'Scifresh' orchard and about 7 km to the 'Royal Gala' orchard block and the Wakatu Lower Moutere weather station was about 5 km from the 'Braeburn' orchard block. The recorded weather data for both stations were obtained from the Hortplus Limited MetWatch database (http://www.hortplus.metwatch.co.nz/index.php). Rain gauges were also placed in the various orchard blocks and the amount of rainfall for each orchard block was recorded in millimetres for comparison with the online records.

RESULTS

The monthly frequencies of rainwater trap and glass slide changes are shown in Table 1. The length of the dry period between rain events affected the number of conidia and ascospores released during subsequent rain. Fewer spores were released with increasing number of non-rainy days per month (Figure 1), while more spores were released with more frequent rainfall.

Rainwater traps in 'Scifresh' and 'Royal Gala' orchard blocks

Rainfalls occurred every month of the year, with the highest rainfall totals of 299.4 and 298.8 mm in the months of June and April 2014 respectively, and the least being 6.4 mm in January 2015 (Figure 1). Mean monthly temperatures for the trapping period ranged from 6.8 to 19.1°C (Figure 1). Fewer conidia and ascospores were collected in rainwater traps with increasing periods of non-rainy days prior to a rainfall event ≥2 mm (Figure 1). The conidia characteristic of *N. ditissima* found in the rainwater traps were long with multiple septations although some were found to be one-celled (Booth 1965). The ascospores found were always two-celled and oval in shape. Overall, more conidia (70.8%) than ascospores (29.2%) were found in the rainwater traps. The seasonal trend in conidial release was very similar between the two orchards. Numbers of conidia released tended to be greatest in autumn (April–May) and lowest in spring (September–October) of both years (Figure 1). This coincided in a general way with frequency and amount of rainfall, except that there were rainy periods in spring with few conidia, e.g., from September to early October 2013, and November to December 2014. Ascospores were observed sporadically in the rainwater traps, with little similarity between orchards and no consistent seasonal pattern over the 2 years.

Although most of the selected cankers in both orchards produced visible sporodochia and perithecia that released conidia and ascospores, respectively throughout the trapping period, some cankers did not. Not all the cankers released conidia or ascospores at every rainfall event, e.g. no conidia or ascospores were trapped in both orchards in November 2014, even though it rained. The low rainfall (6.4 mm) in January 2015 was associated with no ascospore trapping in either the 'Scifresh' or 'Royal Gala' orchards that month although conidia were trapped. The total number of conidia or ascospores trapped each month was independent of the number of trappings and the total monthly rainfall. For example, in both April and June 2015, four

Table 1 Number of rainwater trap collections and glass slide exposures that began in each of the 26 months during the *Neonectria ditissima* inoculum study in three orchard blocks: rainwater traps were set up in 'Royal Gala' and 'Scifresh' blocks and glass slides were installed in the 'Royal Gala' block until June 2014 and continued in the 'Braeburn' block thereafter. Exposure periods were started in advance of rainy period ≥2 mm rainfall.

Month	2013	2014	2015	2013	2014	2015
	Rainwater traps			Glass slides		
January		5	2		6	2
February		2	3		1	2
March		0	3		1	6
April		7	4		7	4
May	6	6	3		6	3
June	5	5	4	5	8	6
July	4	3		6	4	
August	7	3		10	5	
September	7	4		6	5	
October	5	1		6	5	
November	2	4		2	6	
December	3	5		3	4	
	39	44	19	38	58	23
		Total 103			Total 119	

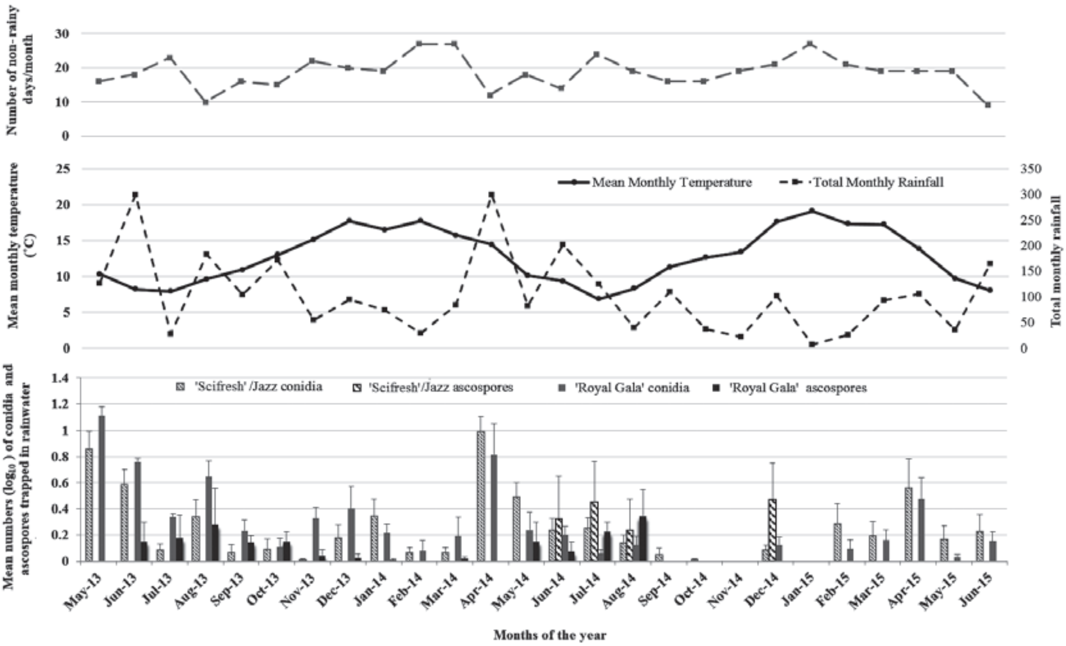

Figure 1 Rainwater traps: Mean monthly temperature (°C) and total monthly rainfall for each month during the trapping period, the number of non-rainy days per month and the mean numbers (Log_{10}) of conidia and ascospores in rainwater traps from two Motueka apple orchard blocks ('Scifresh' and 'Royal Gala') from May 2013 to June 2015. Weather data were from the New Zealand Institute for Plant & Food Research Limited Riwaka weather station. Bars represent standard errors of the monthly mean ascospore and conidium numbers.

rainwater trap collections were made in each of the months (Table 1), but conidia trapped were highest in April 2015 and less in June 2015 (Figure 1). However, from June to October 2013, the number of conidia declined despite regular rainfalls.

Vaseline®-coated glass slide traps in 'Royal Gala' and 'Braeburn' orchard blocks

In both orchards, the slides trapped no spores in weeks without rainfall, showing that rainfall was a requirement for both ascospore and conidium release and transport to the slide surface. In the 'Royal Gala' block (June 2013–June 2014), both conidia and ascospores were trapped at any time that rainfall was frequent. Both spore types peaked in August 2013 and January and April 2014 and ascospores only peaked in October 2013 (Figure 2). The greatest numbers of ascospores were trapped in April 2015. There was no conidia or ascospores trapped in November 2014. In January 2015, ascospores were trapped but no conidia. In the 'Braeburn' orchard, rainfall occurred every month with the highest amount being 102.8 and 100.8 mm in the months of June 2014 and April 2015 respectively, and the least being 7.3 mm in January 2015 (Figure 2). The mean monthly temperatures for the trapping period ranged from 7.3 to 17.7°C (Figure 2). Fewer conidia or ascospores were trapped on the

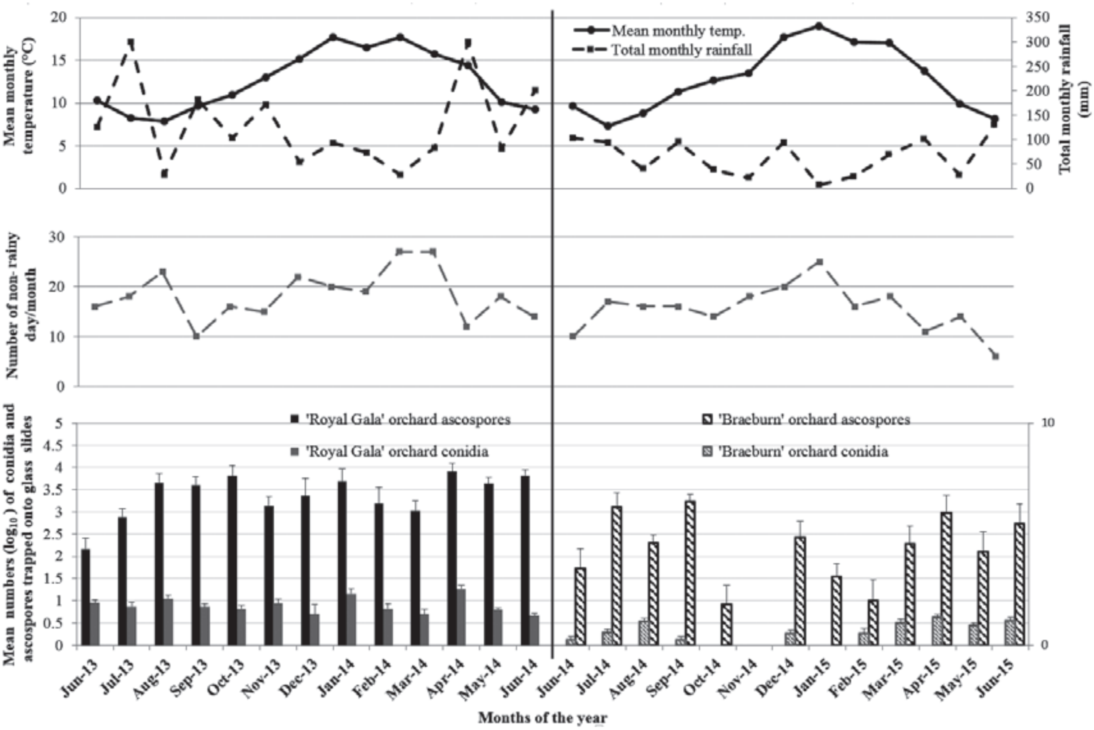

Figure 2 Glass slide traps: Mean monthly temperature (°C) for each month during the trapping period and total monthly rainfall, the number of non-rainy days per month and the mean numbers of (Log_{10}) of conidia and ascospores trapped on Vaseline®-coated glass slide from two Motueka apple orchard blocks ('Royal Gala' from June 2013 to June 2014 and 'Braeburn' from June 2014 to June 2015). Weather data for the 'Royal Gala' orchard was from the New Zealand Institute for Plant & Food Research Limited Riwaka weather station and the weather data for the 'Braeburn' orchard was from Wakatu weather station, Lower Moutere. Bars represent standard errors of the monthly mean ascospore and conidium numbers.

glass slides when longer periods of non-rainy days preceded rainfall event ≥2 mm (Figure 2). Conidia and ascospores were trapped from both orchards in most months of this study, with peak ascospore numbers observed in July and September 2014 and then in April and June 2015 in the 'Braeburn' orchard (Figure 2). Overall, more spores were trapped in the 'Royal Gala' orchard than the 'Braeburn' orchard (Figure 2). Not all the canker lesions released ascospores or conidia at every exposure time, even though it may have rained on or before the day the slides were collected. Overall, more ascospores (98%) than conidia (2%) were trapped on glass slides. Both spore types readily germinated within 18 hrs on glass slides.

In April 2014, when greater numbers of conidia and ascospores were trapped, glass slides placed randomly within the 'Royal Gala' orchard away from canker sources captured more conidia (80 conidia/mm^2) than ascospores (20 ascospores/mm^2) but both spore types were in very low numbers compared with those captured on the slides that were fixed close to stem cankers (<200 conidia/mm^2 and >1000 ascospores/mm^2).

The five slides collected and replaced on five occasions in the 'Royal Gala' orchard while still raining or drizzling did not capture any ascospores but only a few conidia (<20 conidia/mm^2). These slide changes were discontinued after five rainfall events (data not included).

Statistical analyses

There were significant correlations between mean ascospore numbers and total monthly rainfall (r=0.712; P=0.006) on the glass-slide traps in the 'Braeburn' orchard (June 2014 to June 2015) but conidia in the 'Braeburn' orchard, and ascospores

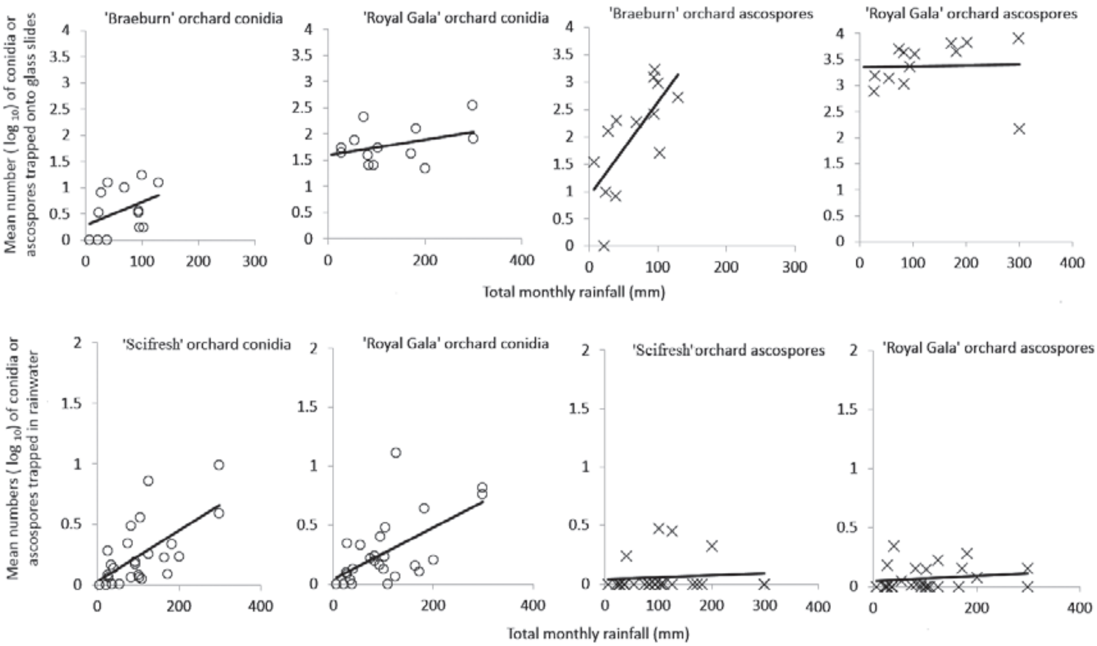

Figure 3 Regression relationships between total monthly rainfall and conidia or ascospores trapped on Vaseline®-coated glass slides from two Motueka 'Braeburn' and 'Royal Gala' orchards, respectively and conidia or ascospores trapped in rainwater from two Motueka 'Scifresh' and 'Royal Gala' orchards, respectively. Weather data for the 'Royal Gala' orchard was from the New Zealand Institute for Plant & Food Research Limited Riwaka weather station and the weather data for the 'Braeburn orchard was from Wakatu weather station, Lower Moutere.

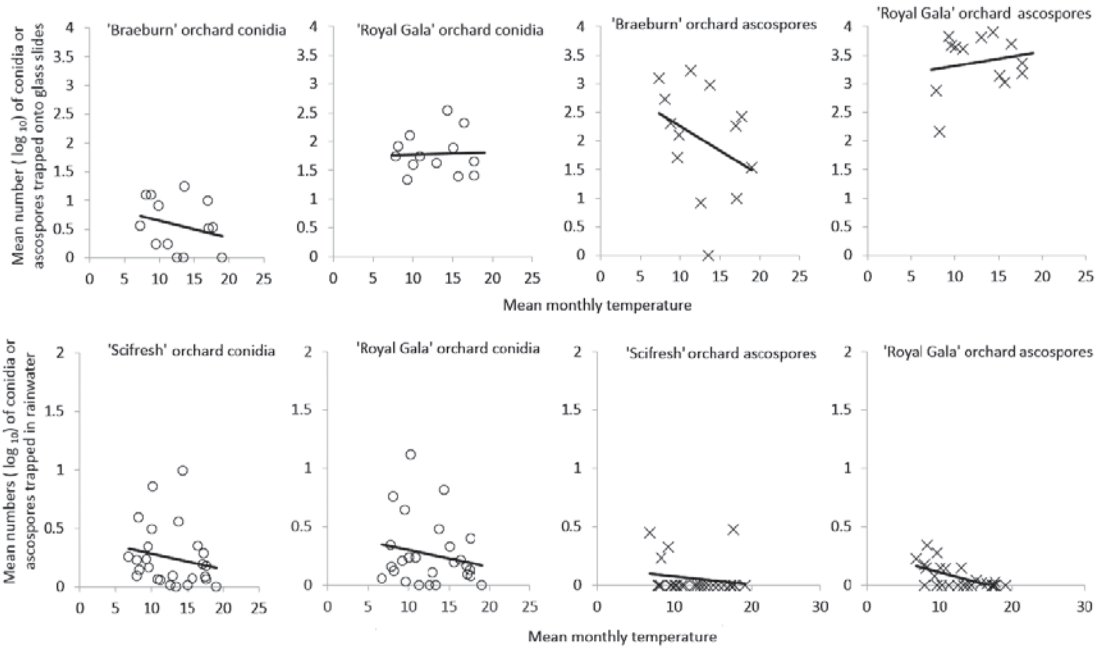

Figure 4 Regression relationships between mean monthly temperatures verses conidia or ascospores trapped respectively, onto Vaseline®-coated glass slide or into rainwater traps installed in Motueka 'Braeburn', 'Royal Gala' and 'Scifresh' orchards, respectively. Weather data for the 'Royal Gala' orchard was from the New Zealand Institute for Plant & Food Research Limited Riwaka weather station and the weather data for the 'Braeburn orchard was from Wakatu weather station, Lower Moutere.

numbers in the 'Royal Gala' orchard, respectively, were not significantly correlated with rainfall (Figure 3). There was a significant correlation between mean conidia trapped and rainfall in both the 'Scifresh' (r =0.652; P<0.001) and 'Royal Gala' (r =0.646; P<0.001) orchards with the rainfall traps, but ascospore numbers were not significantly correlated with rainfall (Figure 3). There was a positive correlation between conidia numbers trapped and the monthly rainfall totals. However, there was no correlation between spore numbers and individual rainfall events. There was a significant correlation between mean ascospores trapped on glass slides and temperature in the 'Royal Gala' orchard (r=-0.615; P<0.001) but no significant correlation between mean conidia or ascospores trapped onto glass slides or rainwater traps and temperature in the 'Scifresh', 'Braeburn' and 'Royal Gala' orchards (Figure 4).

DISCUSSION

This study shows that in the Tasman region of New Zealand both conidia and ascospores can be produced all year round when there is rainfall, irrespective of the season. This finding is consistent with Weber (2014) who reported that conidia and/or ascospores are available all year round in Germany, except for very dry periods in summer and freezing conditions in winter. Although dry periods can occur during summer in New Zealand, prolonged periods of freezing temperatures in winter do not occur in the Tasman region. In both years of the study, there were also periods in spring to early summer when few conidia were trapped, despite frequent rainfall.

The longer-term variation in numbers of trapped spores within the sampled orchards could have been caused by variation in the amount of sporulation in cankers in the vicinity of the traps, as influenced by cultivar susceptibility and disease management. However, the small numbers of conidia found in rainwater traps in the year 2014–15 compared with 2013–14 may have been partly related to different factors. For example, the lower number of conidia trapped in winter and spring in both the 'Royal Gala' and 'Scifresh' orchards was possibly as a result of lower temperatures between 6.4 and 8.3°C in winter and probably fungicide applications to control *Venturia inaequalis* in spring which are part of scab management in apple orchards. Although fungicides may play a role in spore production, the 'Braeburn' orchard had no fungicide application and therefore lower spore numbers from the 'Braeburn' verses the 'Royal Gala' orchard could have been due to difference in cultivar, rainfall events or disease management. More research is required to determine the effect of fungicides on spore production. Fluctuations in spore numbers could also have been due to other factors, for example, rainfall causing more spore release, or heavy rain diluting available spores, making heavier rain events less associated with high numbers of spores or longer periods of non-rain events exceeding 3–5 days may have accounted for the fluctuations in spore release. The conditions required to induce *N. ditissima* conidia and ascospore production *in planta* are not well understood.

The availability of both spore types during fruit picking and leaf fall is of great concern as both picking wounds and leaf scars are susceptible to infection (Amponsah et al. 2015). Picking wounds were found to be the main source for new canker infections in New Zealand orchards (Campbell et al. 2016). The number of spores captured at any month of the year would probably be enough to cause infection, as only a few spores are required for infection in New Zealand (Walter et al. 2016). Inoculum control is, therefore, critical and needs to be undertaken all year round.

Glass slides collected more ascospores than conidia and rainwater traps collected more conidia than ascospores. The trapping efficiency of either type of trap was not determined, but the choice of which trapping device required for future spore trapping in orchards may depend on the location of the canker lesion. The capturing of both types of spores on glass slides placed randomly in the orchard and away from canker sources indicates that both spores can be airborne and rain dispersed. The ascospores captured were mature when released as they were found to be viable, germinating on the glass slides or water in the rainwater traps. In contrast, Ransom (1997) reported that, in Australia, European canker was reported to be present at Spreyton in northern Tasmania for a period of ≈40 years, from almost 20 years prior to 1954 when an eradication programme commenced until it was last detected in 1974. However, the ascospores never reached maturity. Beresford & Kim (2011) reported that weather data near Spreyton showed that the rainfall frequency and temperature thresholds were just exceeded in April and May (autumn) and in October and November (spring) and it appeared that conditions around Spreyton were generally unfavourable for European canker development.

The five slides collected while it was still raining did not capture any ascospores, suggesting that ascospores were released only after rainfall, and not during rainfall. It is possible that ascospore release occurs when lesions imbibe water, and/or that perithecia need to dry slightly after rain before they release ascospores. The latter was shown by Lortie & Kuntz (1963) who reported that *N. galligena* (now *N. ditissima*) "ascospores were discharged as wetted perithecia began to dry out" and shrink in diameter, suggesting that both "wetting and drying are important factors in ascospore discharge". In many of ascomycete species, ascospores are expelled from the ascus, due to the generation of turgor pressure within the ascus (Hallen & Trail 2008). After rain, the shrinking of the perithecial wall against the turgid asci may contribute to the discharge of ascospores. More research is required to confirm whether ascospores are released primarily after the rain has stopped. However, it is well known that perithecia can also exude ascospores in cirrhi when cankers have been exposed to high humidity for a few days (Weber 2014). The very few ascospores that were seen in rainwater traps might have been washed from such cirrhi, rather than expelled from perithecia during rain.

In conclusion, EC spores are available all year round in New Zealand as long as there is rainfall. The release of both conidia and ascospores occurred during periods when rainfall was frequent, as shown by both rainwater traps and glass slides. There tended to be large numbers of conidia and ascospores produced in April–May, but peaks occurred at other times as well. Ascospore release did not follow any specific seasonal pattern, however apple orchard cultural practices through disease management and tree growth physiology makes it possible for wounding of all kinds (pruning, leaf scar, fruit picking, bud scale wind damage etc.) to occur. This, coinciding with the Tasman weather which has no extremes (i.e. too hot or too cold) makes the apple tree highly susceptible to EC infection any time of the year.

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REFERENCES

Amponsah NT, Walter M, Beresford RM, Scheper RWA 2015. Seasonal wound presence and susceptibility to *Neonectria ditissima* infection in New Zealand apple trees. New Zealand Plant Protection 68: 250–256.

Beresford RM, Kim KS 2011. Identification of regional climatic conditions favorable for development of European canker of apple. Phytopathology 101: 135–146.

Booth C 1965. IMI Descriptions of Fungi and Bacteria, 1965, No 15, pp Sheet 147. CAB International, Wallingford, UK.

Campbell RE, Roy S, Curnow T, Walter M 2016. Monitoring methods and spatial patterns of European canker disease in commercial orchards. New Zealand Plant Protection 69: 213-220.

CPC 2005. CAB International Crop Protection Compendium Globule Module. Commonwealth Agricultural Bureau International, Wallingford, United Kingdom.

Hallen H, Trail F 2008. The L-Type calcium ion channel Cch1 affects ascospore discharge and mycelial growth in the filamentous fungus *Gibberella zeae* (anamorph *Fusarium graminearum*). Eukaryotic Cell 7: 415-424.

Lortie M, Kuntz JE 1963. Ascospore discharge and conidium release by *Nectria galligena* Bres. under field and laboratory conditions. Canadian Journal of Botany. 41: 1203-1210

McCracken AR, Berrie A, Barbara DJ, Locke T, Cooke LR, Phelps K, Swinburne TR, Brown AE, Ellerker B, Langrell SRH 2003. Relative significance of nursery infections and orchard inoculum in the development and spread of apple canker (*Nectria galligena*) in young orchards. Plant Pathology 52: 553–566.

Ransom LM 1997. The eradication of *Nectria galligena* from apple trees in Tasmania, 1954–1991. Australasian Plant Pathology. 26:121–125.

Swinburne TR 1971. The seasonal release of spores of *Nectria galligena* from apple cankers in Northern Ireland. Annals of Applied Biology 69: 97–104.

Walter M, Roy S, Fisher BN, Mackle L, Amponsah NT, Curnow T, Campbell RE, Braun P, Reineke A, Scheper RWA 2016. How many conidia are required for wound infection of apple plants by *Neonectria ditissima*? New Zealand Plant Protection 69: 238–245.

Weber RWS 2014. Biology and control of the apple canker fungus *Neonectria ditissima* (syn. *N. galligena*) from a Northwestern European perspective. Erwerbs-Obstbau 56: 95–107.

Wilson EE 1966. Development of European canker in a California apple district. Plant Disease Report 50: 182–186.

Xu X-M, Butt DJ 1996. Tests of fungicides for post-germination activity against *Nectria galligena*, causal agent of canker and fruit rot of apple. Crop Protection 15: 513–519.