Resilient Forests Research Programme

SCION FORESTS PRODUCTS INNOVATION

Identifying the key pathosystem components of red needle cast

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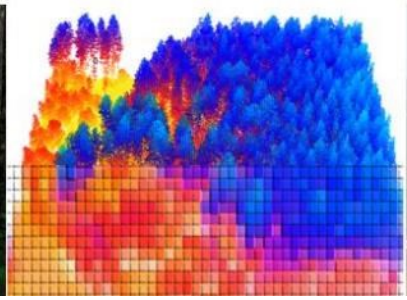


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FOREST GROWERS RESEARCH

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EXECUTIVE SUMMARY

Red needle cast (RNC), caused by *Phytophthora pluvialis*, is a threat to New Zealand's Radiata pine forests. Infected pine foliage becomes red and is prematurely cast from the tree. In cases of heavy and repeated defoliation, tree growth can be reduced. Development of a process based epidemiological model, which allows prediction of disease outbreaks, could be an important tool for risk assessment and RNC management. To develop such a model, a sound understanding of the epidemiology of the *radiata pine-pluvialis* pathosystem and the impact of pathogen, host and environment on each disease component (underlined below) is required.

In this report, we will describe the key components of the RNC disease cycle: infection, pathogen growth (latent/incubation period), sporulation, spread, needle discolouration and defoliation, and pathogen survival. We then outline the current state of understanding on how components of RNC are influenced by pathogen, host and environment. For each component, we review whether:

i) adequate information is available,
ii) some information exists but more is required, or
iii) no useful information is available.

In cases where insufficient knowledge is available for development of an epidemiological model, recommendations are made for assessment of components.

The first component of the *radiata pine-pluvialis* pathosystem is infection. Sporangia or zoospores of *P. pluvialis* disperse to the primary host tissue; needles, via water splash, and are assumed to accumulate at the base of the needle fascicle due to the predominance of symptoms seen here. Zoospores encyst on the needle, germinate, and produce hyphae that enter the needle. Following infection, is the growth stage, in which *P. pluvialis* establishes within the needle. The relationship between the timing of sporulation and symptom expression and the order in which they occur is unknown. However, current understanding suggests that *P. pluvialis* sporangia emerge from the stomata of needles on green tissue (sporulation), followed by the discolouration of the needle from green to red in distinct lesions starting at the point of infection. The growth stage may be referred to as the latent or incubation period. The latent period is the time between infection and sporulation. The incubation period is the time between infection and lesion formation. It is uncertain if the discolouration of the needle is caused by *P. pluvialis* or if this is a host response. Sporangia or zoospores are then locally dispersed by water splash to nearby needles resulting in polycyclic infections (spread). Fascicles which contain needle lesions are cast from the tree (defoliation), possibly as a host response, thus halting the infection cycle. The production of sexual structures (oospores) is possible by *P. pluvialis*, however, this has not been observed within pine needles. The survival mechanism of *P. pluvialis* between epidemics, and the primary inoculum source is unknown.

The impact of pathogen, host and environmental factors on the *radiata pine-pluvialis* pathosystem components of infection, pathogen growth (latent/incubation period), sporulation, spread, discolouration, defoliation, and survival have not been studied in depth (Figure 1). Moisture and temperature are thought to be key drivers of infection and sporulation with outbreaks peaking in winter and with minimal detection over summer months. Light may also impact various components (sporulation, spread and discoloration); however, all evidence for the influence on light on disease stages is currently from other *Phytophthora* pathosystems. Host factors such as needle and tree age as well as stand structure likely influence spread, however, these variables are closely linked, so there is uncertainty to the magnitude of their individual impact. Host resistance of Radiata pine to *P. pluvialis* has been observed as a heritable trait, indicating that host genotype can affect RNC development, however, the stage in which this resistance occurs is uncertain. Although we have indications that these factors may influence various RNC pathosystem components, the evidence is largely anecdotal or based on other *Phytopthora* systems.

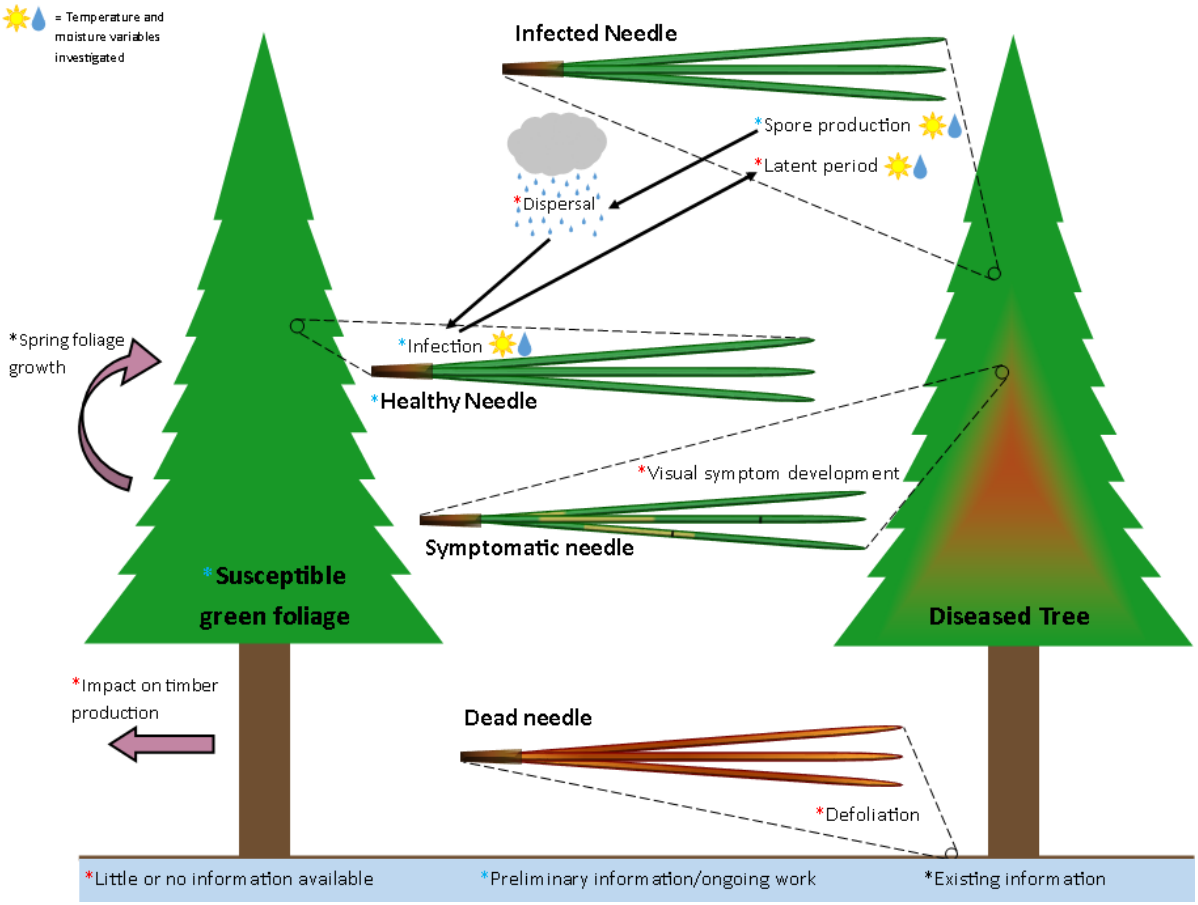

Figure 1. The disease cycle of RNC. Healthy susceptible needles become infected under conditions of moderate temperatures and high rainfall. Following a period of growth, *P. pluvialis* begins to sporulate and the needle discolours from green to red in distinct lesions. Spores are spread to other green needle tissue to start secondary infections via water splash. Discoloured needles are cast from the tree and are removed from the disease cycle. There is little or no information for each stage of this cycle, but work is underway for some stages such as infection and sporulation.

Some information exists on the RNC pathosystem, however, insufficient data is currently available to support the development of an epidemiological model. Current methods of studying the impacts of host and environment on RNC under controlled conditions are often unsuitable for model development, as they omit key stages such as discolouration and defoliation. We recommend the first steps for assessing the pathosystem components of RNC in radiata pine is to develop an intermediate detached twig method, which undergoes all stages of the infection cycle. This approach is not constrained by the time and size limitations of using whole plants. This assay can then be used to assess the impact of key host and environmental factors. We suggest that temperature and moisture should be the first factors considered. As these may impact pathosystem components in a component dependant manner, experiments should be broken up to examine the individual effect of temperature and moisture separately on infection or growth, sporulation and discolouration. The interaction between these environmental factors may also be important and should be eventually studied. In conjunction with controlled experiments, field monitoring of RNC outbreaks and driving factors should be undertaken. These field studies will confirm results of lab experiments and aid in calibration of the resulting epidemiological model.

INTRODUCTION

Red needle cast (RNC) of radiata pine, caused by *Phytophthora pluvialis*, is a risk to the New Zealand Forestry industry. Under suitable environmental conditions, infected foliage of radiata pine trees can become heavily defoliated which may result in a reduced growth rate (Beets, McKinley, Oliver, Pearce, & Bulman, 2013). No optimised operational disease management for RNC is currently practiced in New Zealand. This may be due to the unpredictable nature of the disease resulting in uncertainty about the risks of outbreaks. Prediction of RNC through the development of an epidemiological model may aid RNC management by supporting decision tools in deciding when and where management is required. In order to develop a process based epidemiological model for RNC, further understanding is required on the *radiata pine-pluvialis* pathosystem components and quantification on how pathogen, host and environmental factors may influence the rate of transition between each pathosystem component (Figure 2).

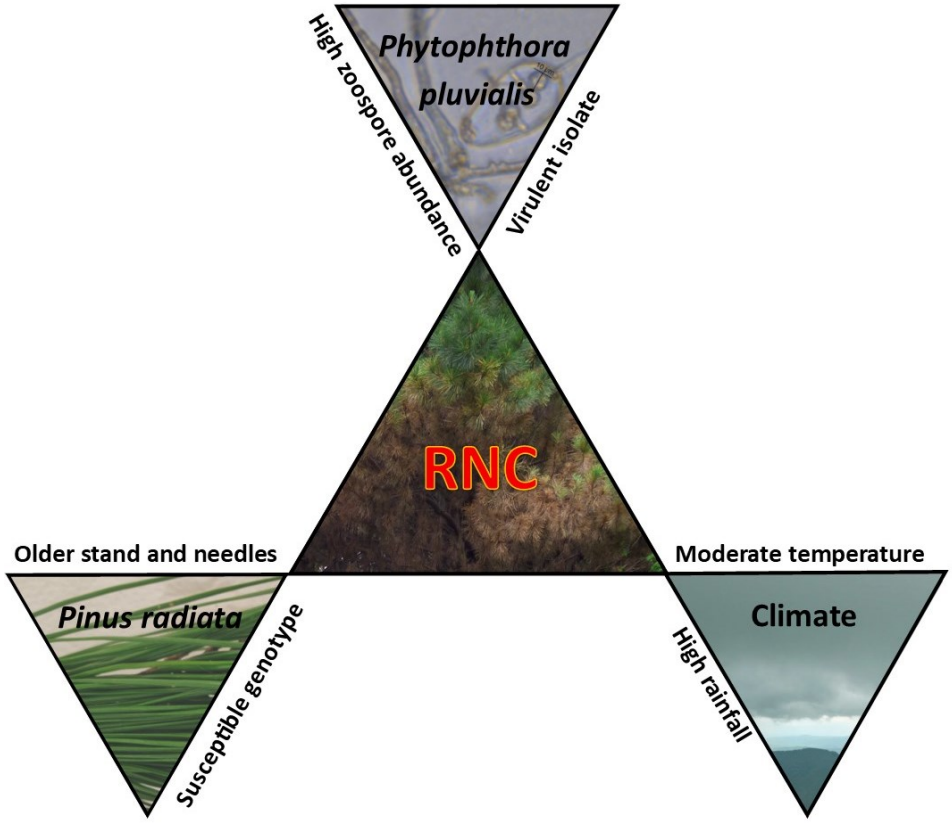

Figure 2. The disease triangle, a paradigm of pathology. Disease will only occur when a virulent pathogen, susceptible host and conducive environment are present.

In this report, we will describe the key components of the RNC disease cycle: infection, pathogen growth (latent/incubation period), sporulation, spread, needle discolouration and defoliation, and pathogen survival. We then outline the current state of understanding on how components of RNC are influenced by pathogen, host and environment. For each component, we review whether:

i) adequate information is available,
ii) some information exists but more is required, or
iii) no useful information is available.

A proposed model for RNC in the field is given. This expands on the base model for RNC created as part of the Healthy Trees, Healthy Future Programme. In cases where insufficient knowledge is available for development of the epidemiological model, recommendations are made for assessment of components and examples of experimental plans to provide required data are outlined.

PHYTOPHTHORA LIFECYCLE

Our current understanding of *P. pluvialis* is largely based on the general *Phytophthora* lifecycle (Figure 3). *Phytophthora* species asexually reproduce through sporangia. Sporangia may directly germinate or indirectly germinate through the release of mobile zoospores (Judelson & Blanco, 2005). Depending on species and environmental conditions, either sporangia or zoospores can act as diaspores (dispersal stage). Zoospores are presumed to be the main dispersal and infection propagule of *P. pluvialis* (Gomez-Gallego, Gommers, Bader, & Williams, 2019), as sporangia have been described as only partially caducous (i.e. they only detach occasionally). Upon reaching a host, zoospores encyst and germinate (Judelson & Blanco, 2005). Many *Phytophthora* are hemibiotrophic, which means they will initially colonise the host as a biotroph feeding on living tissue without actively killing the host before switching to a necrotrophic stage in which the pathogen feeds on dead tissue (Chepsergon, Motaung, Bellieny-Rabelo, & Moleleki, 2020). It is uncertain if the interaction between *P. pluvialis* and radiata pine needles is hemibiotrophic. Host or environmental stimuli induce reproduction, in which asexual sporangia, or in some cases sexual oospores are produced and cause secondary infection (Judelson & Blanco, 2005). This occurs repeatably whilst host and environmental conditions allow resulting in polycylic disease (Gomez-Gallego, Gommers, et al., 2019).

Many *Phytophthora* species form resting survival structures such as oospores or chlamydospores to survive unfavourable conditions (Chepsergon et al., 2020). *P. pluvialis* also forms hyphal swellings as part of its lifecycle (Reeser, 2013). The purpose of the hyphal swellings is uncertain. Hyphal swellings are often observed prior to formation of sporangia in culture (Banham C, personal communication), so may have a role in the asexual reproductive pathway. Chlamydospores have not been observed in *P. pluvialis*, thus oospores are the only recognised survival structure (Reeser, 2013). Oospores are sexual reproductive spores. *Phytophthora* can be self-fertile (homothallic) where an individual can produce oospores, as is the case for *P. pluvialis* (Tabima et al., 2021), or can require cross fertilisation (heterothallic).

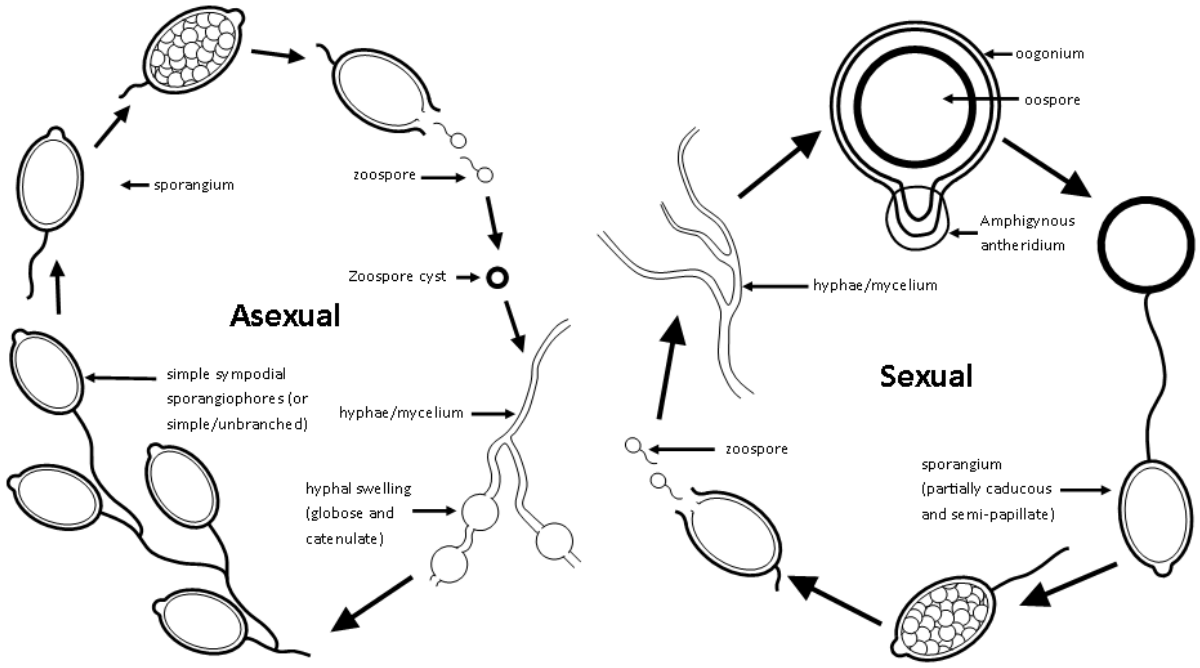

Figure 3. Lifecycle of *Phytophthora pluvialis*. *P. pluvialis* produces asexual sporangia which can germinate directly or indirectly through the release of zoospores. Flagellated zoospores swim within water films to a susceptible host and encyst at a potential infection site. The cyst then germinates through production of hyphae. The hyphae penetrate the host and grow before reproducing asexually or sexually through the production of oospores. Adapted from Abad (2019).

RADIATA-PLUVIALIS PATHOSYSTEM

RNC occurs when the polycyclic lifecycle of *P. pluvialis* repeats within a population of susceptible radiata pine needles under suitable environmental conditions. This pathosystem is made up of the following stages:

1. Infection
Infection is the process in which healthy green needle tissue is colonised by *P. pluvialis*. There is a scarcity of knowledge on the specific processes of infection of radiata pine needles by *P. pluvialis*, with current understanding based on other aerially spread *Phytophthora*, such as *P. infestans*. Zoospores of *P. pluvialis* are dispersed to healthy needle tissue within water films, such as rain droplets, and promptly swim to the needle surface using flagella and taxis, such as autoaggreation or chemotaxis (Chepsergon et al., 2020; Judelson & Blanco, 2005; Walker & van West, 2007). Zoospores then encyst, allowing adhesion to the needle and increased protection through the development of a cell wall (Judelson & Ah-Fong, 2019). The cyst germinates and produces hyphae that penetrates the cuticle to occupy the intercellular space as mycelium (Fawke, Doumane, & Schornack, 2015).

2. Growth
Growth is the stage in which *P. pluvialis* establishes within green needle tissue and consumes host nutrients. Intercellular hyphae within the needle produce haustorium which invaginate the host cell membrane for effector release and nutrient extraction (Chepsergon et al., 2020). Although the needle is infected, there are no visible signs or symptoms of infection on the needle. The growth stage may be referred to as the latent period when indicating the time between infection and sporulation or the incubation when relating to the time to lesion formation.

Early work has been completed to estimate the latent and incubation period of *P. pluvialis* in radiata needles under optimal conditions. Dick, Williams, Bader, Gardner, and Bulman (2014) observed lesions on detached needles within eight days of inoculation when incubated under presumed optimal conditions of high moisture and 17°C. In a similar detached needle assay, Gomez-Gallego, et al. (2019) first observed sporangia on the needle surface from three days post inoculation with a peak in the proportion of sporulating needles at five to seven days post inoculation depending on host resistance (Gomez-Gallego, et al., 2019). Infected whole plants displayed dual peaks in proportion of lesioned needles; first at four and then a larger peak at 20 days post inoculation (Gomez-Gallego, et al., 2019). Infections of whole plants indicated a peak in infection of four days as detected by qPCR (Gomez-Gallego, et al., 2019). Based on infection peaks in detached needle and whole plant assays Gomez-Gallego, et al. (2019) estimated re-infection cycles of 4-6 days. Both these assays were conducted in optimal conditions, so it is likely that development of symptoms in the field will occur at a much slower rate (Dick et al., 2014; Gomez-Gallego, et al., 2019).

3. Sporulation
Sporulation is the production of propagules which have the potential to cause secondary infections. Under persistent needle wetness, sporangia are produced on hyphae that emerge from stomata on green needle tissue at the edge of red lesions (Dick et al., 2014) (Figure 4). The relationship between sporulation and lesion formation (discolouration) is uncertain. Gomez-Gallego, et al. (2019) indicated the necrotrophic stage of *P. pluvialis* may be linked to sporulation with alignment between the incidence of sporangia production and proportion of lesioned needles.

Figure 4. Sporangia of *P. pluvialis* produced on hyphae emerging from stomata in green needle tissue directly ahead of a red lesion

4. Spread

Spread is the transfer of infective propagules from a sporulating needle to a new potential host or another needle on the same host. Water splash is the only significantly recognised method of propagule dispersal of inoculum throughout the canopy (Gomez-Gallego, et al., 2019). Field observations of RNC often appear as connected patches of red needles suggesting water-splash results in a highly localised spread (Figure 5). The requirement for wetness for *P. pluvialis* sporulation, and rain splash for dispersal of zoospores, likely underpins the findings of Fraser et al. (2020) which suggest the number of rain events correlates with RNC detection in forests.

Figure 5. Localised spread of disease, as indicated by red foliage, from the lower canopy to a naturally regenerating understory tree.

5. Discolouration

Discolouration refers to the development of symptomatic red lesions on infected needles (Figure 6). RNC first expresses as distinct bands of pale-olive or khaki lesions on infected radiata pine needles often accompanied by dark resin bands (Dick et al., 2014). It is uncertain if lesions are a host resistance response or directly caused by *P. pluvialis,* however they are commonly used as an indicator of successful *P. pluvialis* infection. Lesions are most frequently observed at the base of needles, next to the fascicle sheath (Dick et al., 2014). The placement of lesions could be due to gravitational accumulation of inoculum suspensions at the needle base, or creation of a favourable microclimate by the fascicle sheath, tightness of needle bundle or proximity to the twig. These early symptoms can only be observed by closely examining a needle and thus cannot readily be detected in a forest by current remote sensing methods or ground scouting.

As the disease progresses and the needles begin to senesce, these lesions turn a burnt red colour (Dick et al., 2014). As patches of needles reach this red stage, the tree foliage takes on a red hue, and can be more readily detected. Timing of symptom development varies between years and sites, but generally foliage discolouration can begin to appear from late autumn (Fraser et al., 2020). As temperatures decrease and rainfall increases, the red foliage coverage increases and spreads across a stand with disease presence generally peaking in winter or spring. RNC is most commonly observed in the lower canopy, but under conducive conditions can extend to the full height of the tree (Dick et al., 2014; Ganley et al., 2014; Gomez-Gallego, et al., 2019). It is likely by the time RNC is easily observable, infections may already be significant.

Figure 6. Typical field RNC symptoms where foliage appears as patches of red needles (a). The underside of the branch is commonly symptomatic (b). On closer inspection, needles have distinct lesions (c) and resin bands (d and e).

6. Defoliation

Defoliation is the removal of symptomatic fascicles from the tree system thus halting potential spread. Discoloured fascicles are readily cast resulting in defoliation of affected trees (Fraser et al., 2020). Casting of the entire fascicle can occur when only one needle within a fascicle begins to display lesions (Dick et al., 2014). Repeated casting events can result in highly defoliated trees by late spring, when new green flush begins emerging (Dick et al., 2014). Defoliation tends to begin in the lower crown but can progress up the crown matching patterns of discolouration (Gomez-Gallego, Williams, Leuzinger, Scott, & Bader, 2020). Defoliation of the lower crown of pine grafts can result in decreased stem diameter, height and above ground biomass in some radiata genotypes (Gomez-Gallego et al., 2020). In severe cases of upper crown or complete defoliation, tree growth may experience greater losses (Gomez-Gallego et al., 2020).

7. Survival

Survival refers to the dormant stage of disease over summer in which symptoms are not generally observed. The mechanism in which *P. pluvialis* survives unfavourable warm dry summers is an enigma. Severely infected needles are cast from the tree, and as new flush emerges, previously "red" trees appear unaffected. The next season, new infections occur with the source of primary inoculum uncertain. The life stage between needle casting and the next season's infection is a major gap in our understanding of the RNC pathosystem. The lack of knowledge of *P. pluvialis* survival presents uncertainty in initial inoculum load for modelling and prediction of outbreaks. *P. pluvialis* does not appear to have a saprobic stage thus an alternative survival mechanism is required. Possibilities include survival as oospores or slow progressing infections in the crown, root or other host tissue. The first step to address this major knowledge gap is to determine whether the entire RNC lifecycle occur on needles in the crown, or if there are some stages, such as oospores, in the soil or plant debris.

PATHOGEN IMPACTS ON RNC

The pathosystem components most impacted by pathogen characteristics are likely infection, sporulation and spread (Table 1). *P. pluvialis* has three known propagules; sporangia, zoospores and oospores. The type of propagule produced and concentration (inoculum load) can influence infection success and dispersal. Propagule type may have a role in survival. Whilst zoospores are short lived, oospores can survive longer periods and may provide answers on the survival component of RNC. The genetic diversity of *P. pluvialis* is low, however, different strains may vary in ability to infect, sporulate and damage the host. The dependence of RNC components on these pathogen traits is discussed below with the level of current knowledge stated and areas which require further investigation highlighted.

Table 1. Summary of evidence of role of pathogen factors impacting each pathosystem component

		Pathogen characteristic		
		Primary inoculum load	Propagule type	Pathogen genotype
Pathosystem component	Infection	Success increases with concentration	Zoospores have a greater infection potential	Higher virulence genotypes have potential to infect a greater range of host genotypes?
	Growth	NA	NA	?
	Sporulation	NA	NA	High fecundity genotypes produce a greater number of spores?
	Spread	NA	Zoospores for short dispersal, possibly sporangia for longer dispersal?	?
	Discolouration	NA	NA	Aggressive isolates may result in increased formation rate or size of lesions?
	Defoliation	NA	NA	?
	Survival	NA	Oospores are best suited for longer survival	?

NA : Pathogen factor is unlikely to influence component
? : Interaction between pathosystem component and pathogen trait uncertain

Inoculum concentration

Inoculum load is a key driver for infection success. The minimum *P. pluvialis* zoospore concentration for consistent infection of needles of *P. radiata* is proposed as 200 zoospores per ml (Ganley, Scott, and Bader ,2015). Higher zoospore concentrations resulted in increased infection frequency (Ganley et al., 2015). This is expected, as probability of infection logically increases with infection attempts. However, some *Phytophthora* zoospores also display auto-aggregation (Ko & Chase, 1973), and it has been suggested, that aggregation of zoospores can aid plant targeting and infection success in a zoospore density dependant manner (Galiana, Cohen, Thomen, Etienne, & Noblin, 2019; Kong & Hong, 2010). Anecdotal evidence suggest *P. pluvialis* may also exhibit chemotaxis, where germinated cysts in sterile pond water were observed to grow towards submerged *P. radiata* needles (Banham C, personal communication), however evidence for auto aggregation has not been observed.

Propagule type

Propagule type can influence infection and spread, with different asexual spores varying in motility and infection potential. *P. pluvialis* produces two types of asexual spore; sporangia and zoospores (Reeser, 2013). *P. pluvialis* sporangia are partially caducous (Reeser, 2013) meaning that sporangia may remain attached to the needle or be released. Mature *Phytophthora* sporangia can germinate directly, or indirectly through release of motile zoospores (Gomez-Gallego, LeBoldus, et al., 2019; Judelson & Blanco, 2005). Zoospores tend to have a greater infection potential over direct germination of sporangia as demonstrated by Widmer (2009) in *P. ramorum*. Due to their

greater motility, zoospores are thought to be the main dispersal agent in *P. pluvialis*. As zoospores have a greater requirement for moisture and tend to be less robust than sporangia (Judelson & Blanco, 2005), direct release and germination of sporangia may have a greater role in unfavourable environments or in long distance dispersal. More research is needed on the conditions which prompt sporangia release or trigger direct/indirect germination to further understand the spread dynamics of *P. pluvialis*.

The survival component of RNC is unknown. *P. pluvialis* sexual spores; oospores, may have a role in survival, however, this has not been studied in a disease cycle context. One hypothesis for survival in unfavourable conditions is through oospores in soil, leaf litter or within the foliage. Although observed in culture, no oospores have been observed within infected *P. radiata* needles (Scott, Taylor, & Williams, 2019). *P. pluvialis* oospores have been shown by vital staining to remain viable, although at low percentages, for at least 3 - 4.5 weeks at temperatures up to 25 ºC on bark (Hood et al., 2014). This would allow for a small percent of exposed oospores to survive for a least one month of the New Zealand summer. In contrast, oospores of other species of *Phytophthora* have been shown to survive much longer time periods, for example Babadoost & Pavon (2013) showed that *P. capsici* oospores survived more than 36 months in sterilised soil placed in field conditions. Although not associated with root disease, *P. pluvialis* has been detected in the rhizosphere of infected *P. radiata* roots (Scott et al., 2019), suggesting the possibility of soil or roots as a niche for survival structures of *P. pluvialis*. If *P. pluvialis* can survive in soil or leaf litter, given the main dispersal method of water splash, primary infection of trees would be expected to occur where foliage is closer to the ground, such as can be the case with edge trees.

Experiments on the survival of *P. pluvialis* as zoospores has also been completed. Scott et al. (2019) indicated a limited infection window of a few hours (without consistent hydration) otherwise zoospores become unviable (Scott et al., 2019). The methods used by Scott et al. (2019) only relate to dried and encysted zoospores. Other species like *P. kernoviae* and *P. ramorum* can survive a wide range of pH and electrical conductivity levels for more than 14 days in diluted Hoagland's solution at 20°C (Kong & Hong, 2010). However, in pure water *P. kernoviae* zoospores could scarcely survive over five days and *P. ramorum* did not survive over three days (15°C) (Kong & Hong, 2010). *In vitro* experiments conducted by Davidson, Rizzo, Garbelotto, Tjosvold, and Slaughter (2002) showed that *P. ramorum* zoospore survival on moist filter paper at 15 ° C averaged less than 20% after 30 days. The ability for *P. pluvialis* zoospores to survive immersed in solution remains untested, however, this is thought to be a moot point, as under favourable conditions the zoospore is a position to encyst and germinate, rather than remain dormant as a survival structure. Due to the need for moisture, zoospores are unlikely to be a long-term survival structure.

Pathogen genotype

The genotype of the *P. pluvialis* strain involved in the RNC interaction may impact rates of all pathosystem components. Strains may vary in number of spores produced (fecundity), range of host genotypes they are able to infect (virulence) or damage they may cause (aggression). There is a relatively low genetic diversity of *P. pluvialis* in New Zealand thus differences between genotypes may not yet be large (Tabima et al., 2021). Ability to infect may also depend on age of the individual organism. *P. pluvialas* virulence has been shown to decrease overtime in mycelium cultures (Gomez-Gallego, et al., 2019). Passaging the isolate through pine needles via zoospore inoculation can revitalise virulence (Gomez-Gallego, et al., 2019). In lab-based studies, mixed inoculations are used to allow for selection of the most virulent isolates (Gomez-Gallego, et al., 2019). There has been some work completed to determine the number and diversity of *P. pluvialis* genotypes present in New Zealand (Tabima et al., 2021), however, no work has been completed on the genetic differences between fecundity, virulence, or aggression.

State of knowledge: some information exists but more is required

There is a reasonable amount of knowledge on the effect of inoculum load on infection. There is much less knowledge on the role of propagule type on the RNC pathosystem. Zoospores have been established as an important part of *P. pluvialis* infection and spread and interact with moisture levels for both these components. There is more uncertainty on the role of sporangia in

disease dynamics and the conditions that favour sporangia release and direct germination over the release of zoospores. Although interesting, for the purpose of model building, overall sporulation can be used to indicate inoculum pressure. Research on caducity of sporangia may be interesting when we increase scope from spread within a tree to spread between trees (Appendix - Experiment 1).

The role of oospores as a survival mechanism has been suggested but not tested resulting in insufficient available information. Experiments designed to determine the mechanism for primary infection in the field would be valuable to further understand the lifecycle of *P. pluvialis* and determine primary inoculum load. It would also provide insight on the transmission pathways, e.g if oospores can persist in soil, and may also aid the development of management techniques. An experiment to determine oospore persistence in soil would provide evidence on whether this is a possible survival mechanism (Appendix - Experiment 2).

HOST IMPACTS ON RNC

Host genotype, tissue, age and nutrient status have been implicated in the infection process, needle discolouration and sporulation (Table 2). Host genotype and tissue are static factors, therefore may be less important for a dynamic model. Tree age and needle age may play a greater role in predicting RNC epidemics, particularly in cases of repeat disease cycles, where older needles have been removed from the system. Our current understanding of the dependence of pathosystem components on host components is discussed, and where additional knowledge is required recommendations for experiments are given.

Table 2. Summary of evidence on the impact of host factors on each pathosystem component

		Host Factor					
		Host Genotype	Host tissue	Tree age	Needle age	Needle density	Needle nutrient status
Pathosystem component	Infection	More resistant genotypes have reduced infection	Needles are primary host tissue	?	Younger needles may be more resistant to infection?	Higher needle density may improve microclimate for infection?	NA
	Growth	?	?	?	?	?	?
	Sporulation	More resistant genotypes have reduced sporulation	Needles only known sporulating tissue	?	?	Higher needle density may improve microclimate for sporulation?	Reduced nutrient availability to the pathogen induces sporulation?
	Spread	NA	?	?	?	Higher density increases the proximity of needles for spread?	?
	Discolouration	?	Needles only symptomatic tissue	Symptoms are commonly observed on older trees	?	?	Lesions may indicate needle sections with reduced nutrients?
	Defoliation	?	NA	?	Older needles are more commonly cast?	?	?
	Survival	NA	Infections of other host tissue e.g. roots may be a survival pathway?	?	?	?	?

NA : Host factor is unlikely to influence component
? : Interaction between pathosystem component and host factor uncertain

Host genotype

Host genotype can result in varying degrees of resistance to *P. pluvialis*. In detached needle assays under optimal conditions, fewer infected pine needles of a resistant genotype reached the sporulation stage and fewer and smaller lesions (discolouration) developed compared to a susceptible genotype (Gomez-Gallego, et al., 2019). (Graham, Li, & Ganley, 2014) also found that lesion size and number varied between host genotypes. Host genotype did not alter infection rate, latent period or incubation period (Gomez-Gallego, et al., 2019).

Host tissue

Host tissue has a major influence on the occurrence of RNC. Therefore, model development will focus on foliage in particular. Other host tissues may have roles in specific components of the RNC cycle such as survival. Whilst the primary host tissue of *P. pluvialis* in *P. radiata* are needles (Dick et al., 2014), it is possible, although uncommon, for *P. pluvialis* to infect *P. radiata* roots leading to fine root loss (Scott et al., 2019). Survival of *P. pluvialis* on other *P. radiata* tissue such as bark and sapwood has been shown to be negligible (Hood et al., 2014). *P. pluvialis* has a larger tissue host range in other species such as Douglas Fir (*Pseudotsuga menziesii*), primarily resulting in needle lesions and casting but also the formation of twig lesions and tip dieback in seedlings (Hansen, Reeser, Sutton, Gardner, & Williams, 2015).

Tree age

Evidence of host age impacts on RNC are largely from field observations of discoloured foliage on older trees. Most evidence is in reference to age effect on discolouration; however, age is also likely to impact on other earlier stages of RNC. Reports of RNC are most common on trees older than four years and most severe on trees over 10 years old (Dick et al., 2014). This may indicate an age-related resistance effect, especially considering younger trees tend to show symptoms only when they are amongst heavily infected older stands (Dick et al., 2014). Alternatively, differences in severity between tree of different ages may be indirectly due to age associated differences in stand structure, such as canopy microclimate, proximity of branches between trees, number of available needles and height from the forest floor. These age-related differences in stand structure may alter suitability of infection conditions and dispersal through proximity of inoculum sources.

Needle age

At a needle level, older needles tend to be more commonly symptomatic with new flush appearing unaffected (Ganley et al., 2014). At face-value, this suggests older needles are more susceptible, however, this may also be a result of the overlap between RNC peak season and the growth of new needles. The RNC cycle tends to end in summer. As red needles are cast, new flushes of needles can occur. Generally, summer conditions are not favourable for RNC infections, and thus this younger flush of needles is unaffected.

Needle density

A higher density crown may increase spread through proximity of needles, as well promote a favourable high humidity microclimate for infection and sporulation. Needle density is a function of other factors including tree age (discussed above), stand management such as pruning/thinning and environment. Needle density can also be impacted by the defoliation component of RNC in which casting of needles results in reduced density. As older needles tend to be cast (discussed in needle age), defoliated trees have both a lower needle density and younger needles. It is uncertain which of these factors, or if a combination of both, may influence the reduced occurrence of repeat disease cycles on the same tree over multiple years. Targeted lab-based studies are required to untangle the direct and indirect effects of tree/needle age and needle density to determine if either has an impact on susceptibility to RNC.

Needle nutrient status

Sporulation and discolouration may be partially dependant on nutrient availability. In controlled studies of *P. pluvialis* infection, zoospore suspensions are produced by rinsing nutrient broth from mycelium suspensions and resuspending in water (Dick et al., 2014; Ganley et al., 2015; Graham et al., 2018; Rolando, Dick, Gardner, Bader, & Williams, 2017). This change from broth to water can result in the production of sporangia within 24 hours (McLay E, personal observation). The water is a mix of sterile deionised water and sterile pond water. In the case of *P. infestans*, starvation (movement of cultures to water agar) induced sporulation, with nitrogen starvation producing the strongest effect (Vu, 2020). The inclusion of pond water in this mix, may indicate a requirement for presence of microorganisms or their biproducts for sporulation. Confirming the need for microbes, *Phytophthora cinnamomi* only sporulated in the presence of non-sterile soil extract, or sterile soil extract supplemented with *Pseudomonas* species (Chee & Newhook, 1965). Relating to sporulation *in planta*, sporangia are often found on the border between green and

lesioned tissue (Dick et al., 2014). Lesions are often a sign of necrotic tissue and may therefore indicate nutrient depleted tissue. As the pathogen exhausts the resources in the needle (lesion), sporulation is induced at the growing front of the hyphae which has entered the bordering green tissue. Our lab observations with *P. pluvialis* does not support this hypothesis, with sporulating needles often showing no visible lesions, particularly at lower temperatures.

State of knowledge: some information exists but more is required

The proposed model uses a needle as a unit and the tree as a system. This means host genotype and tissue are constant through the course of the disease cycle. It is important to know that base resistance may alter between trees of different genotypes, but further research is out of the scope of the current epidemiological model. Of more importance are the drivers for sporulation and discolouration. If links between nutrient availability, lesion formation and sporulation could be established then lesion development would provide a useful proxy for the abundance of sporulation. However, for the scope of determining how host factors influence components of the epidemiological model, sufficient knowledge is present about needle nutrient availability as a driver of RNC.

Field observations present an apparent age-related resistance to RNC; however, it is uncertain if this is a direct or indirect mechanism of age of tree/needle. Tree age is important for forest scale modelling as it may impact spread through needle density, stand structure and introduces the impacts of management. As our proposed model uses the tree as the system, tree age is considered out of scope for this report. Needle age is of greater interest, particularly in cases of repeat infections, where defoliation events result in trees with only young needles in the lower canopy. In these cases, if young needles are more resistant to disease, and spread usually occurs in the lower canopies, then defoliation may increase the overall tolerance of the tree. This presents the reasonably simple research question of; are younger needles more resistant to RNC than older needles for similar aged trees? Age-related resistance could be easily assessed using a detached needle assay (Appendix - Experiment 3).

ENVIRONMENTAL IMPACTS ON RNC

It is well understood that RNC is a seasonal disease which generally peaks in winter (Fraser et al., 2020). Environment, in particular climate, must have a major impact on several pathosystem components (Table 3). Under favourable conditions, such as high rainfall and moderate to low temperatures, RNC can cause dramatic discoloration and defoliation in New Zealand *Pinus radiata* plantations. However, during unfavourable conditions, RNC can be completely absent from a forest in which it has a history of occurring. The key environmental factors which influence RNC are likely temperature and moisture, however other variables such as light may also play a role. Temperature will likely affect most RNC stages including infection, growth, sporulation and discolouration. Moisture is known to impact infection and sporulation, but the effect on other pathosystem components is uncertain. Light is suggested to influence sporulation, and perhaps discolouration but little information is available. The evidence towards the role of these environmental factors, the key knowledge gaps and experiments to address these gaps are discussed below.

Table 3. Summary of evidence on the role of environmental factors on each pathosystem component

		Environmental factor			
		Temperature	Moisture	Light	Wind
Pathosystem component	Infection	? (likely)	Free moisture is required for infection	?	Wind may reduce moisture availability thus reducing infection?
	Growth	15 – 20 °C optimum	?	?	NA
	Sporulation	Temperature may alter propagule type	Free moisture is required for sporulation	Light in other *Phytophthora* induces zoospore release	Wind may reduce moisture availability thus reducing sporulation?
	Spread	?	Water splash is the main dispersal mechanism	High intensity, or UV light may kill spores	Wind may transport spores within water droplets or cast needles to new hosts?
	Discolouration	? (likely)	?	Light quality/quantity may influence needle discolouration	?
	Defoliation	?	?	?	Wind dislodge discoloured needles from the tree?
	Survival	?	?	High intensity, or UV light may kill survival structures?	?

NA : Environmental factor is unlikely to influence component
? : Interaction between pathosystem component and environmental factor uncertain

Temperature

Temperature has been partially parametrised for RNC. Under completely controlled conditions, optimal growth temperature for *P. pluvialis* on carrot agar is between 15-20°C with no growth above 25°C (Reeser, 2013). The lower temperature limit of *P. pluvialis* in culture has not been established. Detached needle assays often control temperature at an assumed optimal of 17°C (Dick et al., 2014; Ganley et al., 2015; Graham et al., 2018; Rolando et al., 2017) but no detailed temperature-growth curve support this optimal. Presence of RNC in a forest environment appears optimal at lower mean temperatures then lab conditions, however this may be related to covariation of temperature and moisture availability. Fraser et al. (2020) found that the detection of *P. pluvialis* in inoculum traps decreased with increasing temperature variables. Inoculum of *P. pluvialis* was not detected when the mean maximum temperature was above 21.6°C. Further controlled lab studies to determine the temperature limits and optima for infection, growth, sporulation, discolouration and defoliation of *P. pluvialis* on needles would be extremely useful for increasing the power of a predictive model.

In some species of *Phytophthora*, temperature impacts the pathway in which sporangia germinate (Judelson & Blanco, 2005). At higher temperatures, sporangia tend to germinate directly, whilst at lower temperatures indirect germination, i.e. the release of zoospores, is more common (Judelson & Blanco, 2005). For example, in many *Phytophthora* species, such as *P. infestans* and *P. sojae*, a cold snap promotes zoospores release from sporangia (Tani & Judelson, 2006; Wang, Jin, Rui, Liu, & Hou, 2018). Although not directly studied, a one hour period at 4ºC is used for *P. pluvialis* zoospore production for detached needle assays, suggesting a cold snap may induce *P. pluvialis* zoospore release (Dick et al., 2014; Ganley et al., 2015; Graham et al., 2018; Rolando et al., 2017). Temperature impact on sporulation pathway may be partially dependant of *P. pluvialis* isolate. Once mycelium cultures are deprived of nutrients, some *P. pluvialis* isolates immediately produce sporangia and release zoospores without external prompts, whilst other isolates would produce sporangia but require a change in temperature and light to induce zoospore release (McLay E, personal observation). Through control of zoospore release, temperature may influence the inoculum availability and propagule type of *P. pluvialis* thus impacting infection and spread.

Temperature may also impact mobility and aggregation of zoospores and therefore infection. *Phytophthora palmivora* zoospore suspensions on a petri dish aggregated at 16º C and slightly at 20ºC, however, when moved to cooler or warmer temperatures (8, 12, 24 and 28 ºC), aggregates dispersed (Ko & Chase, 1973). The implications of temperature dependant aggregation are uncertain. However, it may be an indicator of the suitability of conditions for infection (aggregation) or exploration (dispersal) to more favourable conditions. There is no evidence available on the effect of temperature on *P. pluvialis* zoospore mobility.

Moisture

Moisture availability has been identified as the most important factor promoting infection, sporulation and spread of the RNC disease cycle (Dick et al., 2014; Fraser et al., 2020; Gomez-Gallego, et al., 2019). This is expected given the reliance of several key *P. pluvialis* life stages including sporangia production and zoospore dispersal on moisture levels. Gomez-Gallego, et al. (2019) found that the presence of disease was directly correlated to mean winter relative humidity (in New Zealand on Douglas Fir), subsequently suggesting that active inoculum increases in winter favoured by high relative humidity. Fraser et al. (2020) reported that the probability of detecting *P. pluvialis* using bait traps increased with relative humidity and rainfall in New Zealand. Very few detections were observed in summer and detection peaked in late winter; this fits with the observation that the number of days with rain was the most significant variable promoting inoculum detection (Fraser et al., 2020). Moist conditions are commonly implemented for detached needle assays (e.g. soaked paper towels) and in in continuously moist conditions sporangia can be observed around the stomata (Dick et al., 2014). Furthermore, severe disease is most frequently recorded in sites prone to mist and fog (Dick et al., 2014). The requirement for “prolonged moisture” is an important requirement for detection, but the duration of moisture, particularly as needle wetness, necessary to initiate infection, growth, sporulation, discolouration and spore spread remains uncertain.

Light

Solar irradiance (SI) can have a negative effect on spread of many plant pathogens through death of spores. The effect of SI on *P. infestans* was found to drastically reduce dry sporangia viability to almost zero on sunny days within 1-3 hours depending on the SI dose (Mizubuti, Aylor, & Fry, 2000). However, on overcast days dry sporangia survival was still measurable after 2-3 hours (Mizubuti et al., 2000). In this experiment the sporangia were dry (kept in aerated conditions resting on filter paper) which may have resulted in a reduced tolerance to SI (Mizubuti et al., 2000). In a different study, the survival of *P. infestans* zoospores in surface water was between 2-8 days (depending on the time of year) when exposed to direct sunlight (Porter & Johnson, 2004). In ambient conditions, spores survived between 14-21 days in surface water; however, when soil was added survival became significantly longer (Porter & Johnson, 2004). Currently, there are no experiments investigating the effect of solar radiation on *P. pluvialis* survival.

Light may also have a role as a signal for sporulation induction. Studies on *P. ramorum* and *P. infestans* suggest that production of sporangia or release of zoospores may be partially controlled

by diurnal effects and can be induced by periods of light or darkness (Shakya, Goss, Dufault, & van Bruggen, 2015; Tooley, Browning, & Vinyard, 2020). Although not directly studied, release of *P. pluvialis* zoospores for detached needle studies appears to be induced by one hour of darkness followed by one hour of light in combination with a temperature change (Dick et al., 2014; Ganley et al., 2015; Graham et al., 2018; Rolando et al., 2017). This methodology suggests *P. pluvialis* zoospore release may also be partially controlled by diurnal affects, however, further studies are required. As mentioned with temperature, this is likely dependant on *P. pluvialis* isolate, with some mycelium cultures in sterile pond water producing sporangia and releasing zoospores in darkness (McLay E, personal observation).

Further, anecdotal evidence suggests the possibility that light may influence the symptoms displayed by needles infected with *P. pluvialis* (discolouration). In detached needle assays basic fluorescent tubes of an unknown light quantity and quality are commonly used (Gomez-Gallego, et al., 2019). Although these may provide enough light for growth, they may differ in intensity and quality of the light spectrum compared to direct natural sunlight. Needles infected with *P. pluvialis* in these assays do not display the typical red banding observed in the field, instead changing from a healthy green, to a khaki/olive, then a grey/brown followed by rot/death. It is interesting to note that RNC symptoms on potted trees in the forest understory, in which light is reduced and humidity is increased, needles appear more grey-brown than the typical red foliage seen at full sunlight, thus more closely resembling lab obtained symptoms (Fraser S, personal observation). Although not yet investigated in *Phytophthora* species, light induces the formation of the distinguishing red lesions in pine needles infected with *Dothistroma septosporum* (Shain & Franich, 1981). It may be worth investigating the reason for production of red coloured lesions in *P. pluvialis* infected needles in some environments but not others with a focus on the role of light intensity and quality as well as moisture.

Wind

Phytophthora have a general requirement for moisture (Chepsergon et al., 2020). Wind may decrease infection and sporulation indirectly through drying of foliage. This would have a greater impact of edge trees which are directly exposed to wind, whilst trees protected within the stand may have a greater ability to maintain a humid microclimate. Wind may aid in the spread of disease through transport of propagules in rain droplets or cast fascicles which are still actively sporulating. This is not the case for some *Phytopthora* such as *Phytophthora capsica* in which dispersal of sporangia by wind currents is infrequent (Granke, Windstam, Hoch, Smart, & Hausbeck, 2009). The impact of wind on RNC is likely minimal at a tree scale.

State of knowledge: some information exists but more is required

Environment has been shown to influence most components of RNC. However, insufficient research has been completed for further parametrisation of an epidemiological model. Environmental conditions are often linked e.g seasonal or diurnal patterns of combinations of change in temperature, humidity and light. Below we consider each environmental factor separately, but, impacts on RNC are likely caused by combinations of factors. Quantifying the impact of environment on RNC is not a simple one, and the best approach may be to start with basic experiments and build up complexity of the interaction of environmental factors as we further our understanding of the pathosystem.

The effect of environmental factors on infection, growth and sporulation can initially be completed in detached needle assays. Discoloration, defoliation and spread are difficult to assess under controlled conditions, however, are key to expanding the model to a forest scale. Currently lesions on single needles within a detached needle assay can be measured, however, these are often noted to not represent field symptoms. These assays must be scaled up to a tree level to determine the effect of environment on the rate of lesion formation at a level which is visible at a needle and foliage level as well as the rate of casting of infected needles. Scion currently has access to five incubation chambers, which could fit approximately four young trees each or two growth rooms which could fit up to 30 trees each. Therefore, a compromise would have to be made between sample size per treatment (e.g. temperature) or number of treatments tested per experiment to carry out whole tree experiments. Either option will require time to collect data from

an appropriate number of samples over a range of treatments. Preliminary data could be collected from field trials to give some insight into environmental impact on symptom development, however, for sufficiently detailed data for the development of an epidemiological model at a tree level, further work on laboratory protocols and long term use of available facilities is required. An intermediate method; using detached twigs, may provide an option where environmental conditions can be controlled whilst allowing the convenience of the detached needle assay. Work will be first required to develop such an assay.

An optimal temperature of 17^{o}C has been cited in the literature, however, there is no published growth curve *in planta* to support this or provide lower or upper limits for infection, growth and sporulation. The optimal temperature (or temperature range) for different pathosystem components may not be the same. A series of detached needle assays could be used to assess the impact of temperature separately for infection (Appendix – Experiment 4) then growth and sporulation (Appendix – Experiment 5). Initially, constant temperatures across day and night will be tested, however, given that evidence suggests sporulation/zoospore release may have a diurnal trigger of temperature and light change, this may have to be expanded to use fluctuating day and night temperatures.

The limited information available on the impact of moisture on RNC suggest it is important for infection, sporulation and spread, however, there is very little work done to parameterise this impact to the detail required to develop an epidemiological model. Detached needle assays are the most practical method for studying RNC in completely controlled conditions, however, due to the requirement of free moisture for these assays they are unsuitable in this case. Potted trees have been used in the past for studying RNC, however, the large size of each tree and time for growth can be limiting. Therefore, this work would need to wait until an intermediate such as a detached twig assay has been developed. The key knowledge gaps are: How does needle wetness duration impact infection, growth and sporulation and does this alter other pathosystem components such as discolouration? (Appendix – Experiment 6) And, how do rain events impact spread of disease within a tree (ie. between needles) and between trees? (Appendix – Experiment 7).

Light may impact sporulation, needle discolouration and survival of *P. pluvialis* spores. Measuring the impact of light on RNC would require an investment in light quality and quantity measurement equipment such as a spectroradiometer, and fluorescent light tubes with corresponding filters fitted into existing growth chambers to achieve a range of light qualities such as photosynthetically active radiation (PAR; 400 – 700 nm) and ultraviolet-B (UV-B; 280 – 320nm) wavebands.

The ability of *P. pluvialis* isolates to sporulate readily without light indicates that light fluctuation may be less significant than other potential signals driving sporulation, thus no further research is currently required. The combination of light, moisture and temperature changes that occur during the day may be important for determining the daily timing and duration of sporulation. However, more information on each of these two other individual parameters (moisture and temperature) is required first.

The direct impact of light quality and quantity on survival of external structures, such as spores, may be interesting for long distance dispersal of *P. pluvialis*, and may contribute to the seasonal patterns of RNC observed. Measurement of viability of propagules under different light conditions could be achieved in detached needles measuring infection success (Appendix – Experiment 8). The impact of light on symptom expression would be much more subjective and would best be completed through use of image analysis of detached needles (Appendix – Experiment 9).

Survival of *P. pluvialis* within needles through unfavourable conditions is yet to be studied. It is noted that RNC is less commonly observed during summer, but this has not been attributed to temperature, moisture or light alone. Although we could study these separately, we are uncertain if survival within the crown during unfavourable conditions is possible. To start, it may be best to test the combination of environmental factors which make up summer conditions to determine if infected green needles may be able to harbour the pathogen over the summer months (Appendix – Experiment 10).

PROPOSED RNC FIELD MODEL

Background

Gomez-Gallego et al. (2019) proposed a dynamical systems model for RNC development in a detached needle assay and an adapted version for needles on whole plants. The goal is now to adapt this model for the field in which weather variables change seasonally, and the system is no longer closed. The "system" here is the tree, and this will be later extended to the forest stand. The system is not closed because infected needles eventually defoliate and leave the system and fresh needles grow in spring. Figure 7 shows the current proposed field model, which is based on the commonly used SEIR–type compartmental models (Gilligan et al. 2002). In contrast to the Gomez-Gallego et al. (2019) models we will not be including an equation for free living pathogen since current evidence suggests that the spores do not survive more than one day (see propagule type), and the importance of any long-term survival structure is not known.

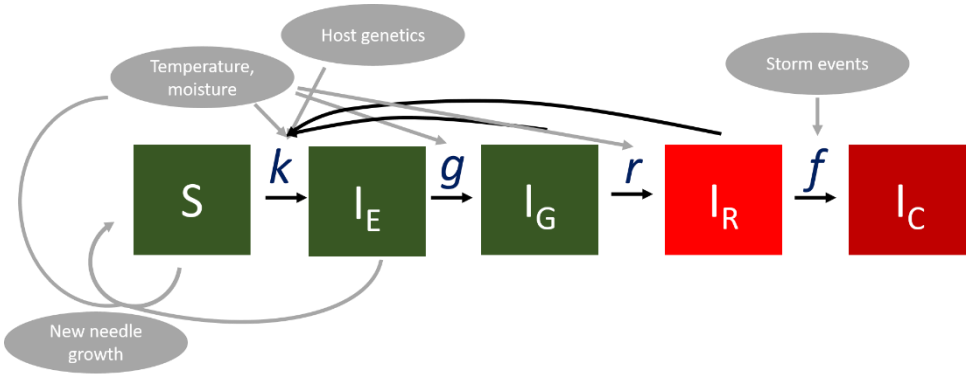

Figure 7. Proposed model schematic for the field epidemiology of RNC. Details on how to model and fit the parts in grey are still to be determined. The key parameters in the proposed model are k – the infection rate, g – the rate of transition from the exposed/latent state to the sporulating state, r – the symptom expression rate, and f – the defoliation rate. k is a function of sporulation rate, spread rate (between needles), and infection probability (given the arrival of spores).

Compartmental type models describe the stages which the needle goes through as discreet stages. This means that even though the transition from a green to red needle is a gradual change, for the purposes of simplicity the model will describe needle symptoms as discrete states (Figure 8). The model will need to be calibrated using field data in which the observer estimates the abundance of symptomatic needles in the canopy visually from the ground. However, initial parameterisation will be accomplished by experiments in the lab. In order to link up lab data to field data we will need to define the symptoms which will classify a needle as a green needle verses a red needle under close observation. For example, a needle may be classified as "red" if at least 50% is discoloured.

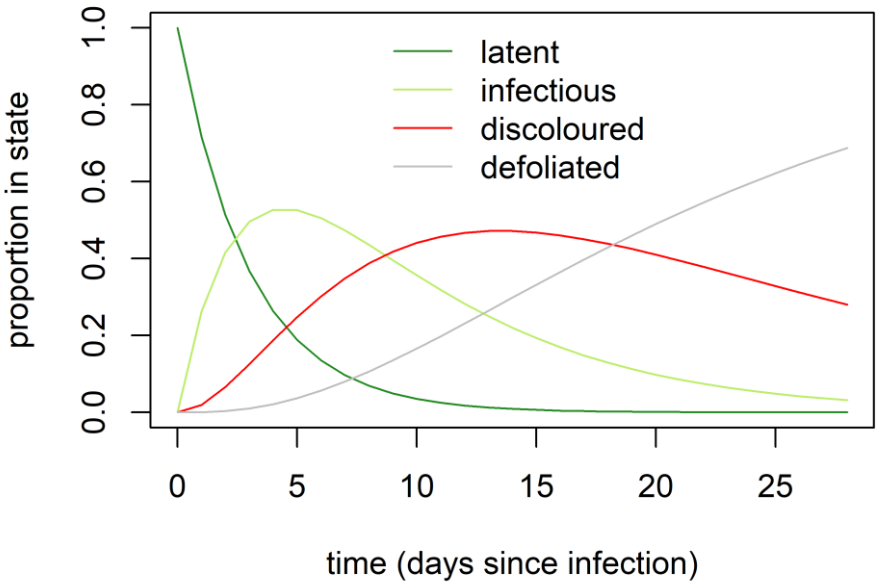

Figure 8. Example of the transition of needles through disease states as described by a system of ordinary differential equations. Proportion of needles in the I_E latent period (infection growing), I_G infectious period (sporulating and still green in appearance to the observer on the ground), I_R discoloured period (no longer sporulating but now visibly discoloured to the observer on the ground) and I_C defoliated (needle has detached from the tree).

Initial discussion suggested that the easiest disease measurement to make in the field at frequent intervals (e.g. fortnightly) was symptoms visible to an observer from the ground. Hence, the field model has the healthy, susceptible needles in one bin and the infected needles split into four bins:

- S – susceptible needles which are not yet infected,
- I_E – needles with an established infection but which are not yet sporulating (this is sometimes called the latent period and can be thought of as the period where the pathogen is growing within the needle but has not yet begun sporulating),
- I_G – infected needles with no visible symptoms (from the ground) but which are sporulating,
- I_R – infected needles with visible symptoms and are no longer sporulating, and
- I_C – cast/defoliated needles.

Note that the current proposed model does not use a time delay as was used in the Gomez-Gallego models, but we can switch to this in the future if it seems more suitable. Additionally, this modelling framework assumes that the infectious sporulating stage is separate from the visually symptomatic stage once the needle discolours and is visible to the observer from the ground. This assumption will be checked during in lab experiments.

We can express the model shown in figure 6 with the following system of differential equations:

$$\frac{dS}{dt} = -kSI_G + growth$$
$$\frac{dI_E}{dt} = kSI_G - gI_E$$
$$\frac{dI_G}{dt} = gI_E - rI_G$$
$$\frac{dI_R}{dt} = rI_G - fI_R$$
$$\frac{dI_C}{dt} = fI_R$$

Field measurements of the total number of needles will not be possible, so the unit for the needles will be the projected area as calculated from scaled images. The size of a section of the projected area of a tree will not be precisely proportional to the volume of the section, or directly proportional to the density per square meter of ground space. However, for the initial modelling we will assume that the projected area is a good enough proxy of density, within the measurement error. Also, S, I_E, and I_G will not be distinguishable from field measurements.

There are four model parameters which will be functions of weather variables, and perhaps needle density:

- k – infection rate, takes into account the rate of sporulation, spread and infection, and is dependent on temperature and moisture.
- g – rate of transition from the latent state to the infectious state (i.e. the sporulating state).
- r – rate of transition from the green sporulating needle (I_G) to the red non-sporulating needle (I_R).
- f – Rate of defoliation/casting.

The above parameters (some of which are actually functions of weather variables) will be parameterised using lab and field experiments. The potential dependency of model parameters is summarised in Table 4. Note that k is a function of spread, sporulation and infection, which in turn are functions of temperature and moisture. Since it was not possible to use known concentrations of inoculum in the lab during experiments on the temperature and moisture dependence of infection these experiments only give the relative effect. k will be modelled as follows:

$$k = \kappa f(m)g(m, temp)h(m, temp)$$

$f()$, $g()$, and $h()$ are functions giving the relative effect of moisture (m) and temperature (temp) on spread, sporulation and infection respectively. These functions will be informed by lab data. κ is a constant multiplier which will be fitted once the model is fitted to field data. It may be possible to simplify the equation for k if the temperature and moisture dependence of each component are aligned.

Table 4. Potential dependency of model parameters on biotic and abiotic factors. The x symbol means that there is a hypothesised dependence. Any dependence with a question mark is unknown and there are currently no plans to inform these relationships. * indicates that lab experiments have been completed, ^{P} indicates that lab experiments have been planned to facilitate parameter estimation. The two factors hypothesised as key drivers of the seasonal dynamics are highlighted in grey.

		Contributing factor								
		Host Genotype	Host tissue	Tree age	Needle age	Needle density	Needle nutrient status	Temperature	Moisture	Light
Pathosystem component	Infection	x	?	?	?	?		x*	x^{P}	?
	Growth	?	?		?			x*	?	?
	Sporulation	?				?	?	x	x^{P}	?
	Spread				?	?			x	?
	Discolouration	?			?		?	?	?	?
	Defoliation			?			?	?	?	?
	Survival		?	?	?					?

Temperature and moisture are the two variables hypothesised to be driving the seasonal disease dynamics alongside defoliation and regrowth. Needle density may have a strong effect on spread rate; however, the initial modelling will assume that this is captured by the $-kSI_G$ term in the model. For the purposes of simplification, any dependency marked with a question mark in Table 4 will be assumed to be negligible. Wind and host genetics are likely to be influential, however we will assume in the initial model that they have no effect.

The parameter estimates from initial lab and field experiments will then be used with further field data to calibrate the model, and finally the model will be validated with field data not already used in model development. The model can then be used to explore potential disease management scenarios and parameter sensitivity. If the model captures the key components of the RNC system, it can be used to understand the underlying disease drivers and in what circumstances to expect an outbreak.

Discrepancies between the model behaviour and field observations can be used to identify alternative frameworks or further factors which might be useful if included in the model. Future modelling could include more sophisticated stochastic models and spatial models to account for the spread of RNC from tree to tree throughout the forest.

CONCLUSIONS

We have a basic understanding of the epidemiology of RNC in radiata pine forests. Current knowledge is based largely on anecdotal data, with insufficient information on all pathosystem components to quantify the drivers of disease (Table 5). The pathosystem components are defined as infection, pathogen growth (latent/incubation period), sporulation, spread, needle discolouration and defoliation, and pathogen survival. Our current level of knowledge indicates that environmental conditions, with an emphasis on temperature and moisture are likely to have the greatest impact on the RNC disease cycle. Other factors such as light, host age, needle density, host genotype, and pathogen propagule type may influence various pathosystem components, however, to a lesser extent to that of temperature and moisture. There has been very little work published on *P. pluvialis* and many assumptions are based on other *Phytophthora*. Most controlled studies have been completed on detached needles thus removing the defoliation, spread and survival stages of the cycle. Field studies capture these three stages, However, they are impractical for the detailed studies required (needle as a unit) for the development of models. A detached twig assay may allow measurement of all pathosystem components under lab conditions. Using a variety of detached needle, detached twig, whole plant assays, and field studies, additional information on the drivers of RNC can be measured. These experiments should in time substantially move forward our understanding of epidemiology of RNC and allow the development of a process based epidemiological model, which allows prediction of disease outbreaks based on pathogen, host and environmental factors.

Table 5. The state of knowledge on how pathosystem components are impacted by pathogen, host and environment where: ✓ = Adequate information is available, - = Some information exists but more is required, or × = No useful information is available.

		Contributing factor		
		Pathogen	Host	Environment
Pathosystem component	Infection	✓	×	-
	Growth	-	×	×
	Sporulation	-	-	-
	Spread	-	-	-
	Discolouration	×	×	-
	Defoliation	×	×	×
	Survival	×	-	-

RECOMMENDATIONS

The objectives of this report are to highlight the key drivers of RNC, identify where information is lacking and make recommendations to address these knowledge gaps. Overall, this document identifies the research priorities to gain sufficient data to improve the predictive power of the epidemiological model. Temperature and moisture were highlighted as key drivers of most stages of the RNC pathosystem, and therefore should be the priority factor for further research. As we will build knowledge on RNC over the course of the research, we recommend starting with simple experiments using known techniques. As understanding of the RNC system increases, these experiments will increase in complexity. For this reason, we recommend first using detached needle assays to determine the temperature curves for infection, growth, sporulation and discolouration (Appendix - Experiment 4 & 5). During this process methods can be developed for measurement of these components as well as parameterising the general *Phytophthora* lifecycle for *P. pluvialis.* During this time, efforts should be made to develop detached twig assays focusing on the ability to measure sporulation, discolouration and defoliation and the timing of these stages in relation to each other. This detached twig assay may allow the measurement of the impact of moisture as duration of needle wetness or wetting events on pathosystem components (Appendix – Experiment 6). Of a lower priority, it may also be worth considering examining the impact of needle age to improve understanding of repeat infections (Appendix – Experiment 3). We also recommend work to further understand the survival stage of RNC (Appendix – Experiment 10) as this will advise transmission routes and primary inoculum loads. In conjunction with controlled experiments, field monitoring of RNC outbreaks and driving factors should be implemented. These field studies will confirm results of lab experiments and aid in calibration of the resulting epidemiological model.

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APPENDICES

Appendix: Example experimental outlines to fill knowledge gaps in the understanding of *radiata-pluvialis* pathosystem

Experiments are listed in the order in which they occurred in the main body of text. Research priority for improvement of the RNC epidemiological model is to first quantify the impact of temperature (Experiment 4 and 5) followed by moisture (Experiment 6) on infection, sporulation, discolouration and defoliation. Research priorities after this point will depend on the findings and methods developed through these experiments. If temperature and moisture data allow for accurate prediction of RNC at a tree level, then research can focus on expanding the model to include spread within a stand over multiple seasons. This expansion would require research into needle age (Experiment 2) for understanding of repeated defoliation, impact of rain on spread (Experiment 7) and survival of *P. pluvialis* between RNC outbreaks (Experiment 10). If temperature and moisture provide insufficient information to improve predication of RNC, then further research at a tree level is required and research would instead be conducted on propagule type (Experiment 1) and light (Experiment 8 and 9).

Experiment 1

How readily do sporangia detach from a sporangiophores and how is this controlled by environmental conditions?

This is an important question that should be addressed; however, methods are not currently developed for measuring sporangia and zoospore release.

Experiment 2

Can *P. pluvialis* persist in the soil as oospores over summer, capable of germinating and infecting plant material?

1) Inoculate sterile soil with *P. pluvialis* oospores within mesh bags and bury 30cm deep outdoors over summer
2) At 0, 1, 2, 4, 8, 12, 16, and 20 weeks post inoculation, place a subset of soil bags in trays and flood with sterile pond water. Float healthy *P. radiata* needles on the water surface and incubate trays at a 14-hour photoperiod with a day/night temperature of 15/5 ^{o}C.
3) At 7-day intervals, attempted isolation on needle baits and replace with fresh healthy needle baits

Experiment 3

Are younger needles more resistant to RNC than older needles for similar aged trees?

1) Inoculate sets of *P. radiata* needles aged either 1, 2, or 3 years with *P. pluvialis* zoospore suspensions for 24h at 17^{o} C in darkness
2) Place needles on moist paper towels on plastic trays. Seal tray with gladwrap and incubate at 17 ^{o}C and a 14h photoperiod.
3) Check a subset of needles from each temperature daily for presence of sporangia and lesions
4) Measure lesion length and number of sporangia on a subset of needles from each temperature at 7, 10- and 14-days post inoculation

Experiment 4

How does temperature effect *P. pluvialis* infection success?

1) Place sets of detached *P. radiata* fascicles in a *P. pluvialis* zoospore suspension for 24 hours at either 2, 5, 10, 17, 20 or 25 ^{o}C in darkness
2) Following inoculation, surface sterilise fascicles by wiping with 70% ethanol, then lay on moist paper towels on plastic trays. Seal tray with gladwrap and incubate at 17^{o}C and a 14h photoperiod for an additional two days

3) Following incubation, surface sterilise fascicles by placing in 70% ethanol for 30s followed by two rinses in sterile distilled water for 30s each. Cut each fascicle into 1-3mm pieces and place individually into wells of a 96 well plate on ice.
4) Send plate to Slipstream for detection of *P. pluvialis* by qPCR

Experiment 5

How does temperature impact on *P. pluvialis* latent period and sporulation severity in detached needles?

1) Inoculate detached *P. radiata* fascicles in a *P. pluvialis* zoospore suspension for 24 hours at 17°C in darkness
2) Place needles on moist paper towels on plastic trays. Seal tray with gladwrap and incubate at either 5, 10, 17, 20 or 25 °C and a 14h photoperiod.
3) Check a subset of needles from each temperature daily for presence of sporangia and lesions
4) Measure lesion length and number of sporangia on a subset of needles from each temperature at 7, 10- and 14-days post inoculation

Experiment 6

How does needle wetness duration impact infection, latent period and sporulation and does this alter other pathosystem components such as discolouration and defoliation?

1) Inoculate *P. radiata* needles on detached twigs by submerging in a *P. pluvialis* zoospore suspension within a Ziplock bag at 17°C for 24 hours
2) Rinse excess spores from twigs, hang within individual horizontal glass jars and incubate at a 14h day/night cycle of 20/10 °C
3) Submit twigs to a range of wetting/drying routines including a combination of:
   a) Misting frequency: e.g daily, biweekly or weekly
   b) Drying frequency: e.g 2h, 6h post misting or not dried
4) Observe needles for the formation of lesions (size and colour) and sporulation (number sporangia). Harvest the run-off from twigs and bait with healthy needles.

Experiment 7

How does rain duration and frequency impact the spread dynamics of RNC within and between trees?

1) Inoculate lower branches of potted tree and surround by healthy trees and place outdoors undercover
2) Expose sets of trees to 5 irrigation regimes
3) Measure weekly total tree RNC and defoliation %, Number of trees infected, distance within and between trees of spread.

Experiment 8

Do UV-B and visible light impact *P. pluvialis* infection success?

1) Place sets of detached *P. radiata* fascicles in a *P. pluvialis* zoospore suspension for 24 hours at 17 °C and combinations of PAR light (0 – 100 µmol m^{-2} s^{-1}) and broadband UV-B light (0 – 2 µmol m^{-2} s^{-1}).
2) Following inoculation, surface sterilise fascicles by wiping with 70% ethanol, then lay on moist paper towels on plastic trays. Seal tray with glad wrap and incubate at 17°C and a 14h photoperiod for an additional two days
3) Following incubation, surface sterilise fascicles by placing in 70% ethanol for 30s followed by two rinses in sterile distilled water for 30s each. Cut each fascicle into 1-3mm pieces and place individually into wells of a 96 well plate on ice.
4) Send plate to Slipstream for detection of *P. pluvialis* by qPCR

Experiment 9

Do UV-B and visible light impact symptom expression of *P. radiata* needles infected with *P. pluvialis* ?

1) Place sets of detached *P. radiata* fascicles in a *P. pluvialis* zoospore suspension for 24 hours at 17 ^{o}C and darkness.
2) Place needles on moist paper towels on shallow plastic trays. Trays are left unsealed to allow UV-B penetration and watered frequently to maintain moisture levels and incubated at 17^{o}C. Chamber photoperiod was 14 hr with the light comprising of combinations of PAR light (0 – 100 µmol m^{-2} s^{-1}) and broadband UV-B light (0 – 2 µmol m^{-2} s^{-1}).
3) Images are taken at 1 hour prior to dusk at 7, 10- and 14-days post inoculation in uniformly light conditions and the colour palette of needles extracted with image analysis.

Experiment 10

Can *P. pluvialis* survive within needles during unfavourable summer periods?

1) Inoculate potted trees with *P. pluvialis* under ideal disease conditions
2) At 4 DPI confirm the presence of *P. pluvialis* within a subset of needles through isolation/qPCR then move trees to standard summer conditions [increase temp, decrease RH. Incr. photoperiod]. Leave a subset of trees within ideal disease conditions as a control.
3) At 10, 17, 24, and 31 DPI, confirm the presence of *P. pluvialis* within a subset of needles through isolation/qPCR then move a subset of trees back to ideal disease conditions and observe for characteristic disease symptoms such as the development of lesions or resin bands.