

Distribution of Swiss needle cast in New Zealand in relation to winter temperature

J. K. Stone^{A,E}, I. A. Hood^B, M. S. Watt^C and J. L. Kerrigan^{A,D}

^ADepartment of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.

^BEnsis Forest Biosecurity and Protection, Private Bag 3020, Rotorua 3046, New Zealand.

^CEnsis, PO Box 29237, Christchurch 8540, New Zealand.

^DPresent address: Department of Entomology, Soils, and Plant Sciences, Clemson University, Clemson, SC 29634, USA.

^ECorresponding author. Email: stonej@science.oregonstate.edu

Abstract. *Phaeocryptopus gaeumannii*, the causal agent of Swiss needle cast disease, is widely distributed throughout New Zealand, where the disease may cause significant growth losses in Douglas-fir (*Pseudotsuga menziesii*) plantations. In western Oregon, where the pathogen is native, pathogen abundance and disease severity are correlated with mean daily winter temperatures and spring moisture, enabling the development of climate-based disease prediction models. The distribution of *P. gaeumannii* and severity of Swiss needle cast was surveyed in 16 Douglas-fir plantations throughout New Zealand in 2005. Retention of foliage was assessed in the field and samples of 1- and 2-year-old needles were collected for assessment of *P. gaeumannii* abundance. Foliage retention and abundance of *P. gaeumannii* varied across sites. Less colonisation by *P. gaeumannii* and greater needle retention was found in the South Island. Abundance of *P. gaeumannii* was found to be positively correlated with August minimum temperature and June average temperature, and showed a similar relationship to winter temperature as observed in western Oregon. These data will be used to derive a disease prediction model for Swiss needle cast in New Zealand that can be used to guide further research and provide short- and long-term disease risk predictions and cost/benefit analyses.

Additional keywords: *Mycosphaerella*, *Pseudotsuga menziesii*.

Introduction

Phaeocryptopus gaeumannii is a foliage-infecting ascomycete that occurs naturally in indigenous Douglas-fir (*Pseudotsuga menziesii*) in western North America. However, the fungus was initially discovered and described from diseased Douglas-fir plantations in Switzerland in 1925, and soon afterward reported from other locations in Europe (Boyce 1940). Infection by this fungus frequently results in chlorotic foliage, defoliation and growth reduction, a condition known as Swiss needle cast (Boyce 1940). Molecular phylogenetic studies have shown that the fungus that causes Swiss needle cast is unrelated to the type of the genus *Phaeocryptopus* and should instead be classified in the genus *Mycosphaerella* (Winton *et al.* 2007). Although a nomenclatural change based on these findings is anticipated, in the present paper the name *P. gaeumannii*, which is well established in the literature, will be used for the causal agent of Swiss needle cast.

Since the discovery of the fungus in Europe, it has been subsequently reported from many parts of the world, and Swiss needle cast has been a serious concern wherever Douglas-fir is cultivated as a forestry species outside of its native range in western North America. Over the past 60 years, outbreaks of Swiss needle cast in forest plantations have occurred in Europe

and the British Isles (Boyce 1940; Peace 1962), the north-eastern USA (Morton and Patton 1970), Turkey (Temel *et al.* 2003), Australia (Marks *et al.* 1982) and New Zealand (Beekhuis 1978; Hood *et al.* 1990). The fungus does not sporulate on dead needles, is not seedborne, and Douglas-fir, including 'bigcone' Douglas-fir (*P. macrocarpa*) in New Zealand (Gadgil 2005), is its only host (Stone *et al.* 2007). However, it can occur as inconspicuous or endophytic infections in living foliage (Hood 1982). Because *P. gaeumannii* does not persist or sporulate saprophytically, it may have become distributed worldwide through movement of infected Douglas-fir nursery stock.

P. gaeumannii was first found in stands of Douglas-fir in New Zealand near Taupo in 1959 (Gilmour 1966), and is now considered established throughout the country. The present distribution of *P. gaeumannii* in New Zealand is thought to have originated from a single initial introduction in the central North Island, from which it gradually spread throughout the country (Hood *et al.* 1990). The fungus was first detected in the northern South Island in 1969 (Hood and Kershaw 1975), and in the southern South Island by the mid 1980s (Hood *et al.* 1990). Subsequent impacts of the disease have been estimated as an overall reduction in stand volume growth of ~20% (Knowles *et al.* 2001).

Greater local abundance of *P. gaeumannii* in Europe (Merkle 1951; Durrieu 1957) and western North America (Hood 1982; Hansen *et al.* 2000) has been associated with areas having higher rainfall, particularly during late spring, and the high incidence of infection observed in North Island Douglas-fir plantations was attributed to the comparatively high spring rainfall in New Zealand (Hood 1982). However, knowledge remains limited on the spatial distribution of the disease in relation to regional climate, altitude, site variation and latitude. Observation suggests that in some parts of New Zealand, Douglas-fir may still be grown with minimal disease impact and acceptable growth rates.

The pathogen has long been present at endemic levels in western North America, where, until recently, it has been considered an insignificant threat to forest health (Boyce 1940; Peace 1962; Hood 1982). However, an unusually severe and persistent outbreak of Swiss needle cast has been affecting Douglas-fir plantations in western Oregon since the mid 1990s (Hansen *et al.* 2000). The disease currently affects ~150 000 ha of Douglas-fir plantations along the western flank of the Oregon Coast Range (Kanaskie *et al.* 2005) resulting in volume growth losses of 15–50%, depending on disease severity (Maguire *et al.* 2002). The relatively abrupt appearance of Swiss needle cast in Oregon has puzzled pathologists because *P. gaeumannii* had not previously been associated with severe disease in forest plantations. Research carried out in the Oregon Coast Range to investigate factors influencing disease distribution demonstrated that ~80% of the spatial variation in disease severity in 1- and 2-year-old foliage, as measured by the proportion of stomata occluded by pseudothecia, could be explained by two climate variables, winter mean daily temperature and spring cumulative leaf wetness (Manter *et al.* 2005). This work led to the development of a disease prediction model based on

winter temperature alone. Subsequent research has supported the conclusion that winter temperature and spring moisture are useful factors for predicting both annual and longer term trends in spatial distribution of Swiss needle cast in western Oregon (Stone and Coop 2006).

In 2005, research was initiated to develop a climate-based disease prediction model for New Zealand Douglas-fir plantations in order to identify regions where the disease is less severe and where Douglas-fir may become a preferred forestry crop. The potential effect of host genetic variation was controlled by limiting the sampling to three seedlots included in two 1996-planted New Zealand Douglas-fir Research Cooperative trials situated at eight South Island and two North Island locations. However, it was necessary to supplement these with additional stands to ensure a full range of latitudinal and altitudinal variation. Defoliation was assessed in the field on a sample of trees at each site and foliage samples were collected for later estimation of colonisation by *P. gaeumannii* in the laboratory. The objectives of this study were to characterise the spatial variation in disease severity and *P. gaeumannii* abundance in New Zealand, and to determine whether the same climate factors that are correlated with spatial variation in disease severity in western North America are also correlated with disease distribution in New Zealand.

Methods

Study sites

Sampling was undertaken over 7 weeks between 20 October and 20 December 2005 during the peak sporulation period of *P. gaeumannii* (Hood and Kershaw 1975). Foliage retention and *P. gaeumannii* colonisation assessments were made at a total of 16 Douglas-fir plantations selected to represent a wide range in mean annual temperature and annual rainfall (Table 1). Ten

Table 1. Site level description of location, altitude, annual rainfall and average June temperature determined both from long-term averages, and data for June 2004

Site ^A	Latitude (°S)	Longitude (°E)	Altitude (m)	Annual rainfall (mm)	Average June temp. ^B	
					Long-term (°C)	2004 (°C)
Manuka Awa	46.1	169.8	225	819	4.5	5.7
Beaumont	45.9	169.5	495	1005	3.7	5.1
Gowan Hills-SS	45.9	168.0	570	1811	2.9	3.8
Gowan Hills-P	45.9	168.1	300	1084	4.1	5.1
Waipori	45.9	169.9	435	905	3.7	6.8
Hanmer	42.6	172.9	420	1179	4.2	7.1
Golden Downs-SS	41.5	172.7	390	1719	4.8	6.3
Golden Downs-P	41.5	172.8	390	1441	4.9	6.8
Karioi	39.4	175.6	795	1388	4.7	5.5
Puketitiri	39.3	176.5	810	2045	5.4	7.6
Te Waka	39.2	176.6	780	2128	5.6	7.1
Putorino	39.1	176.9	285	1501	8.5	9.3
South Kaingaroa	38.9	176.3	780	1625	4.6	6.7
Tauhara	38.7	176.2	480	1314	6.3	7.7
Kaingaroa	38.3	176.8	240	1471	7.4	9.2
Wairangi	38.1	177.9	720	2085	6.6	7.5

^ASites designated 'SS' correspond to seed source trial sites, those designated 'P' correspond to progeny trial sites shown in Fig. 1.

^BTemperature was determined using temporally and spatially splined values.

sites were 9-year-old progeny trial sites in which seed sources were either arranged in a randomised complete block design (four sites designated as ‘P’, Fig. 1; Table 2) or in uniform groups of the same seed source (six sites designated ‘SS’, Fig. 1; Table 2, (G. Stovold, unpubl. data). An additional six sites were stands of similar age and stand density, but mostly of unknown provenance. Eight sites were located in the North Island and eight were located in the South Island (Fig. 1; Table 2).

In the progeny and seed source trial stands, 20 trees were arbitrarily selected from each of three New Zealand seedlots (900, 904, 906) for foliage retention assessments and foliage sampling at each site. At each of the other locations, 20 trees were arbitrarily selected for sampling. The specified seedlots were derived from parent trees grown in New Zealand whose original provenances were: Fort Bragg, CA (900), Florence, OR (904) and western WA (906) in the USA. Seedlot 900 is

widely planted in young (<20-year-old) Douglas-fir plantations in New Zealand, and was also represented in the southern Kaingaroa site.

Foliage retention

Foliage retention was assessed in the field by the same person at all sites. For each tree, two secondary branches subtended at the 4th (basal) node on the primary-axis of one 5th-whorl primary branch were cut with a pole pruner. Foliage retention was visually estimated using a 0–9 (0 = <10% of needles attached, 9 = >90% of needles attached) scale for each of the four internodes (shoots produced in each of the years 2001–2004). A foliage retention index (*FI*), the sum of the retention scores for all four internodes of each branch was used as a response variable for comparing foliage retention among sites and seed sources. Foliage retention for the Manuka Awa site was not included in the foliage retention

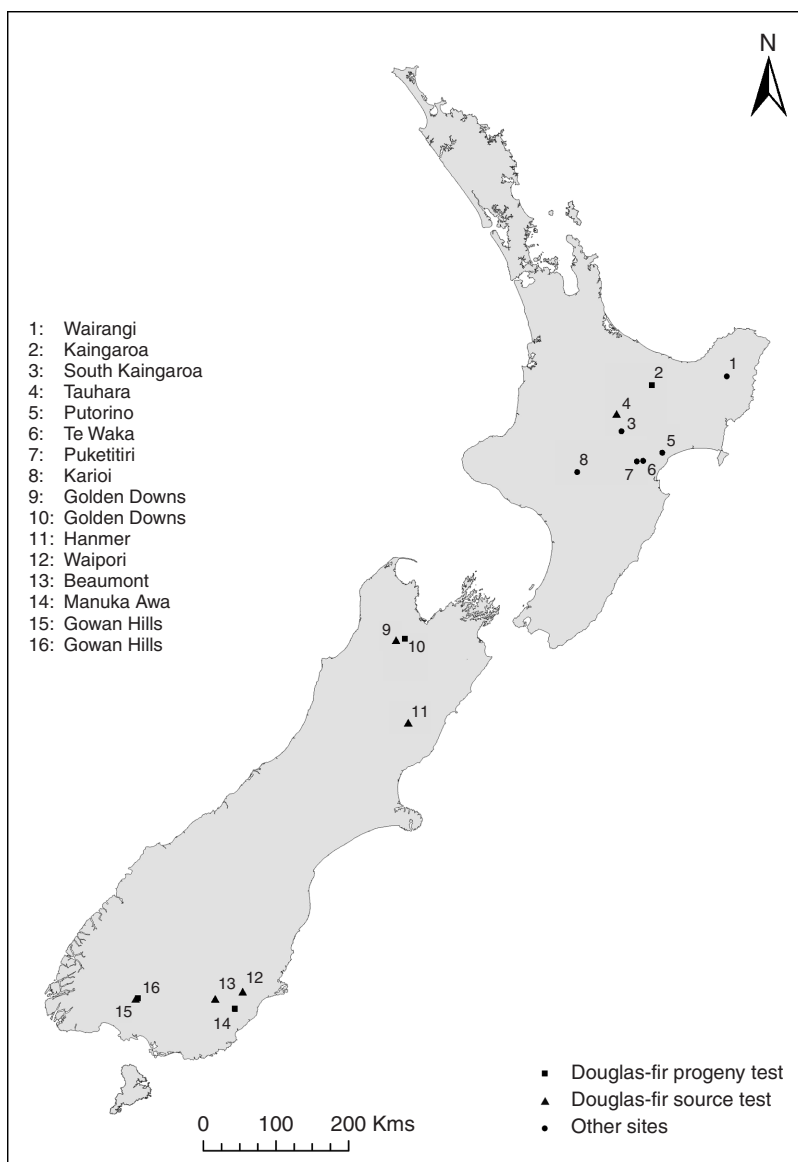


Fig. 1. Locations of sampling sites.

Table 2. Comparison of foliage retention by needle cohort (year produced) among sites

Site ^A	Foliage retention index ^B					Group ^C
	2004	2003	2002	2001	Sum	
Karioi	9.0	9.0	9.0	6.8	33.8	A
Hanmer-SS	9.0	9.0	8.9	6.0	33.0	A
Gowan Hills-SS	9.0	9.0	8.8	4.5	31.2	AB
Golden Downs-P	9.0	9.0	8.7	4.7	31.4	AB
Gowan Hills-P	9.0	9.0	8.5	4.0	30.1	CD
Golden Downs-SS	9.0	9.0	8.4	3.5	30.0	CD
Beaumont-SS	9.0	9.0	8.1	2.7	28.8	D
Kaingaroa-P	9.0	9.0	8.1	0	26.1	E
Waipori-SS	9.0	9.0	7.7	0.4	26.1	E
Tauhara-SS	9.0	9.0	5.5	1.6	25.1	E
S Kaingaroa	9.0	8.7	4.0	0.1	21.8	F
Putorino	8.8	8.8	3.5	0	21.1	F
Wairangi	9.0	8.4	1.6	0	19.0	FG
Puketitiri	9.0	7.8	0.7	0	17.5	G
Te Waka	9.0	8.0	0.1	0	17.1	G

^ASites designated 'SS' correspond to seed source trial sites, those designated 'P' correspond to progeny trial sites shown in Fig. 1. Manuka Awa site is not included here because trees were planted more densely than the other sites, possibly affecting foliage retention.

^BFoliage retention index (*FI*) 0–9 scale where 0 = <10% of needles attached, 9 = >90% of needles attached, i.e. *FI* = 9.0 indicates the full complement of needles retained for a needle cohort. Means of 20 trees per site.

^CMultiple range test Fisher's LSD. Different letters indicate statistically significant ($P = 0.05$) differences among sites.

analysis because trees at that site were planted more densely than the other sites, resulting in more self-shading. A sample of four to six tertiary shoots bearing 2003- and 2004-produced internodes was collected from the same basal secondary branch on each tree and sealed in a labelled polythene bag (one bag per tree). Samples were held at 4°C until processed in the laboratory within 2 weeks of collection.

P. gaeumannii abundance

Foliage samples were returned to the laboratory where the 2003 and 2004 internodes were separated, the needles were removed from branchlets and then pooled by age class. A sample of 50 needles for each age class (2003 and 2004 cohorts) per tree was randomly drawn, needles affixed with the abaxial surface facing upwards to a labelled index card with double sided adhesive tape, and the cards stored frozen (–20°C) until examined.

To estimate abundance of *P. gaeumannii* each 50-needle sample was examined under a binocular dissecting microscope at 40× to determine the proportion of needles bearing pseudothecia (incidence of infection). The first 10 needles on each card with pseudothecia present were then used to determine the proportion of stomata occluded by pseudothecia (pseudothecial density). The needles were examined under a dissecting microscope fitted with a counting grid and the proportion of stomata occluded by pseudothecia in three 2.6 × 0.26-mm segments (base, middle, tip) of each of the 10 needles was determined and averaged. A colonisation index (*CI*), the product of the percent of needles with visible pseudothecia (incidence, $n = 50$) and the average proportion of stomata occluded (pseudothecial density, $n = 10$) was used as a response variable for comparisons of disease severity among sites and seed sources.

Statistical analyses and modelling

Statistical analyses of differences in *FI* and *CI* with site and seed source as independent variables were carried out using PROC mixed with Fisher's LSD multiple comparison procedure (SAS Version 8, The SAS Institute, Cary, NC). The impact of seed source on the relationship between *FI* and *CI* was examined by a mixed model using data from the subset of sites where all three seed sources (900, 904, 906) were present. Two separate analyses related *FI* to *CI* present in 1- and 2-year-old foliage, respectively. ANCOVA was used to determine if slopes or intercepts for these two relationships differed between seed source. Because no significant differences were found, further analyses were conducted on the complete dataset using site level *CI* and *FI* averaged across all seed sources.

CI was correlated with a comprehensive range of climatic variables to identify the main environmental influences on distribution of *P. gaeumannii*. All meteorological data used were long-term monthly averages obtained from thin-plate spline surfaces (Hutchinson and Gessler 1994) fitted to meteorological station data (Leathwick and Stephens 1998). *CI* was normalised by age class, and then averaged to provide a site average, which was unbiased, with respect to needle age class. These average site values of *CI* were then regressed against long-term average rainfall, windspeed, solar radiation, relative humidity, evaporation, and minimum, average and maximum temperature. In addition to the monthly averages, seasonal and annual averages were determined for each variable and used in the analyses.

Two multiple regression models were constructed using these variables to predict *CI*. Initially, data from all sites were included in the model. To ensure that loss of heavily colonised needles did not bias *CI*, and consequently relations between *CI*

and environmental data, a second multiple regression model was constructed that excluded data points from 2-year-old needles (2003 cohort) having a retention score of less than nine. Although the data for the 2-year-old needles were excluded for some sites, the overall normalised site value, using *CI* for the 1-year-old needles was retained, because the retention score for 1-year-old needles (2004 cohort) was never less than nine and the strong correlation observed between *CI* in 1- and 2-year-old needles ($R^2 = 0.78$; $P = 0.0002$) suggested that *CI* for the younger needle age class would provide a useful index for the site. In both multiple regression models, variables were sequentially introduced into the model using the forward selection procedure and retained if inclusion significantly improved the model.

Results

Seed source was not a significant factor in the ANOVA ($P = 0.08$) when foliage retention was compared by seed source for the nine sites where all three seed sources were represented. Consequently, data for the three seed sources were combined for comparisons of foliage retention among sites. Variation in foliage retention across sites was highly significant ($P < 0.0001$). Needle retention varied from ~ 3.7 years at the sites with the least disease to ~ 1.8 years at the most severely diseased sites (Table 2). The entire complement of 2004 needles was retained at all sites, with the effect of disease severity being reflected in partial or complete losses of the 2001, 2002, and 2003 needle cohorts. Severe disease resulted in complete loss of the 2001 cohort at four sites (Puketitiri, Putorino, Te Waka, Wairangi) in the Hawkes Bay region of the eastern North Island, with near elimination of the 2002 cohort at two of these sites (Puketitiri

and Te Waka, Table 2). Greatest retention of older foliage was shown by trees at Golden Downs, Hanmer and Gowan Hills sites in the South Island, and at Karioi in the central North Island (Table 2).

P. gaeumannii was present at all 16 sites sampled. The abundance of *P. gaeumannii* pseudothecia on 1-year-old and 2-year-old needle cohorts varied among sites. Seed source was not a significant factor in the ANOVA ($P = 0.39$ and 0.10 for 1- and 2-year-old needles, respectively) when *CI* was compared by seed source for all sites where three seed sources were sampled. Therefore, data for all three seed sources were pooled for comparisons among sites for all other analyses. The greatest colonisation levels were found in sites in the Hawkes Bay region and central North Island. The greatest colonisation of 1-year-old (2004) needles, $\sim 15\%$ of stomates occupied by *P. gaeumannii* ascocarps, was found at Putorino. About 34% of the stomates of 2-year-old (2003) needles were occupied in the Kaingaroa sample, which also had the greatest overall colonisation for both needle cohorts (Table 3). The higher altitude Karioi site in the central North Island, however, had relatively low colonisation levels, as did the majority of sites in the South Island. The least colonisation was found at Hanmer and sites in the southern South Island, Gowan Hills and Manuka Awa, which had fewer than 1% of stomates of 2-year-old needles occluded by *P. gaeumannii* (Fig. 1; Table 3).

For the subset of sites where all seed sources were present *FI* summed across all needle age classes exhibited a significant negative relationship with *CI* in both 1- ($P = 0.01$) and 2-year-old ($P = 0.02$) foliage. ANCOVA indicated that seed source did not affect either the slope or intercepts for these two relationships (all $P > 0.73$). As seed source did not affect the relationship between

Table 3. Comparison of colonisation index for the 1-year-old (2004), 2-year-old (2003) and sum of both age classes

Site ^A	2004	Group ^C	Colonisation index ^B		Sum	Group ^C
			2003	Group ^C		
Hanmer-SS	0.03 ^C	A	0.37	A	0.40	A
Gowan Hills-SS	0.09	A	0.15	A	0.25	A
Manuka Awa-P	0.67	AB	0.36	A	1.03	A
Gowan Hills-P	1.39	AB	0.51	A	1.95	A
Karioi	0.47	AB	7.71	B	8.18	B
Golden Downs-P	1.71	AB	11.64	BC	13.30	BC
Beaumont-SS	3.77	CD	10.20	B	13.96	BC
Golden Downs-SS	2.22	BC	13.94	CD	15.97	C
Waipori-SS	5.63	E	10.67	BC	16.29	C
Puketitiri	6.71	EF	19.87	DE	25.08	DE
S Kaingaroa	5.01	DE	25.96	FG	30.98	EF
Wairangi	11.18	H	20.23	EF	31.40	EF
Tauhara-SS	10.28	GH	24.04	F	34.32	FG
Putorino	14.53	I	23.08	EF	37.61	FGH
Te Waka	8.15	FG	30.90	GH	39.05	GH
Kaingaroa-P	9.66	GH	33.52	H	43.18	H

^ASites designated 'SS' correspond to seed source trial sites, those designated 'P' correspond to progeny trial sites shown in Fig. 1.

^BThe product of the percent of needles with visible pseudothecia (incidence, $n = 50$) and the average proportion of stomata occluded (pseudothecial density, $n = 10$). Means of 20 sampled trees per site for 2004 and 2003 needle cohorts.

^CFisher's LSD multiple range test. Different letters indicate statistically significant ($P = 0.05$) differences among sites.

FI and *CI*, further analyses across sites used values averaged across all seed sources. Across all sites *FI* was negatively correlated (Fig. 2) with *CI* for both 1- ($P = 0.0007$, $R^2 = 0.60$) and 2-year-old needles ($P = 0.001$, $R^2 = 0.57$).

Univariate relationships showed that *CI* was most strongly related to minimum temperature during August, $T_{m\text{aug}}$, ($P < 0.0001$, $R^2 = 0.71$), and average temperature during June, $T_{av\text{june}}$ ($P < 0.0001$, $R^2 = 0.70$). Both these variables exhibited positive relationships with *CI*. Using multiple regression the best model of *CI* included only $T_{m\text{aug}}$ ($P < 0.0001$, $R^2 = 0.71$). Separating data by year, $T_{m\text{aug}}$ was more strongly correlated (Fig. 3) to *CI* in 1-year-old ($R^2 = 0.82$), rather than 2-year-old needles ($R^2 = 0.49$). Further examination of the relationship in the 2-year-old needles indicated that the low correlation was at least partially attributable to overprediction of *CI* on two of the five sites where the retention score for the 2003 needle cohort was less than nine (see open circles Fig. 3b).

Deletion of *CI* values for sites having 2003 needle cohort retention scores less than nine altered variable selection

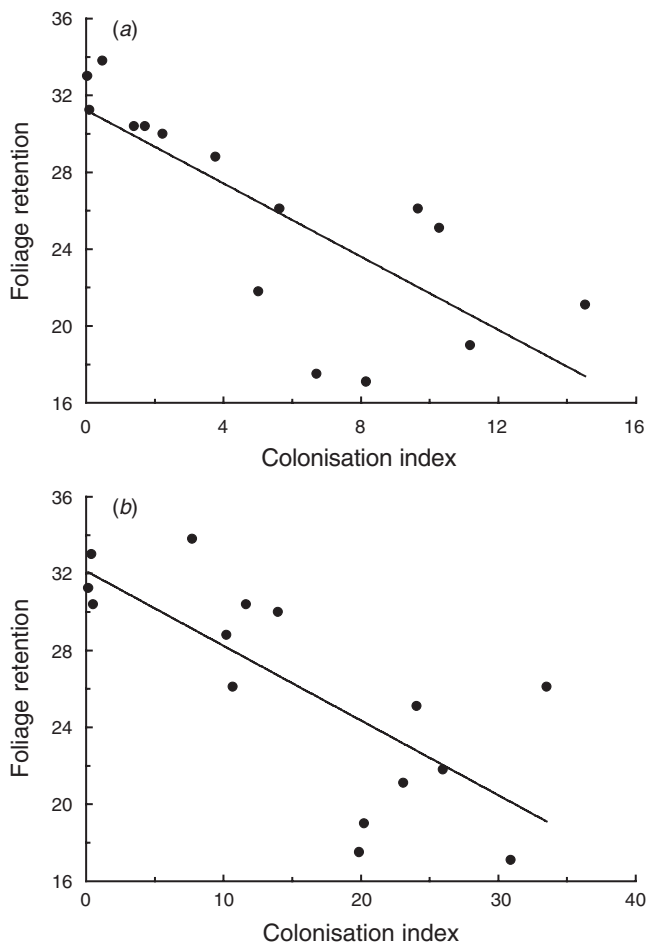


Fig. 2. Relationship between foliage retention ($FI = \text{sum of foliage retention score of } 0\text{--}9$ for four needle cohorts, 2001–2004, see Table 1) and colonisation index [$CI = \text{product of the percent of needles with visible pseudothecia (incidence) and the average proportion of stomata occluded (pseudothecial density)}$] for (a) 1-year old ($P < 0.0007$, $R^2 = 0.60$) and (b) 2-year-old ($P < 0.001$, $R^2 = 0.57$) foliage.

very little, but did improve the overall strength of the relationship for the combined dataset, and for the 2-year-old needles. For this modified dataset, *CI* exhibited the strongest univariate relationships with $T_{av\text{june}}$ ($P < 0.0001$, $R^2 = 0.82$) and $T_{m\text{aug}}$ ($P < 0.0001$, $R^2 = 0.81$). All monthly average minimum temperatures and five of the 12 monthly average temperatures exhibited strong ($R^2 > 0.6$) correlations with *CI* (Fig. 4). For variables other than temperature, moderately strong ($R^2 = 0.40\text{--}0.51$) univariate relationships were also found between *CI* and several monthly solar radiation averages, of which the strongest positive relationship occurred during June ($P = 0.002$, $R^2 = 0.51$). Rainfall, relative humidity, evaporation, and windspeed were only weakly correlated with *CI*.

The final multiple regression model was derived using the modified dataset of *CI* values having corresponding foliage retention scores = 9, and included only $T_{av\text{june}}$ ($P < 0.0001$, $R^2 = 0.82$). No other variables were significant in this model at $P = 0.05$. By age class, $T_{av\text{june}}$ was equally correlated (Fig. 5) to *CI* in 1- and 2-year-old needles ($R^2 = 0.75$). The relationships for 1- and 2-year-old foliage were: $CI = -8.56 + 2.68 T_{av\text{june}}$ (1-year-old needles) and $CI = -24.06 + 7.44 T_{av\text{june}}$ (2-year-old

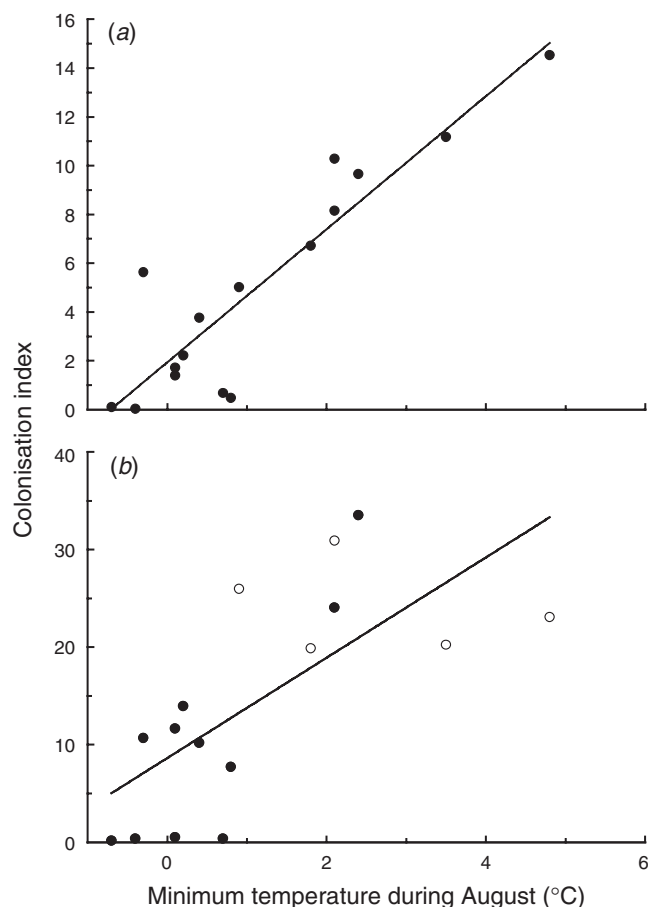


Fig. 3. Relationship between colonisation index (*CI*) and minimum temperature during August for (a) 1-year-old needles ($R^2 = 0.82$) and (b) 2-year-old needles ($R^2 = 0.49$). In (b), sites for which *FI* for the 2003 (2-year-old) needle cohort is < 9 are shown as open circles, whereas those with a *FI* of 9 are shown as closed circles.

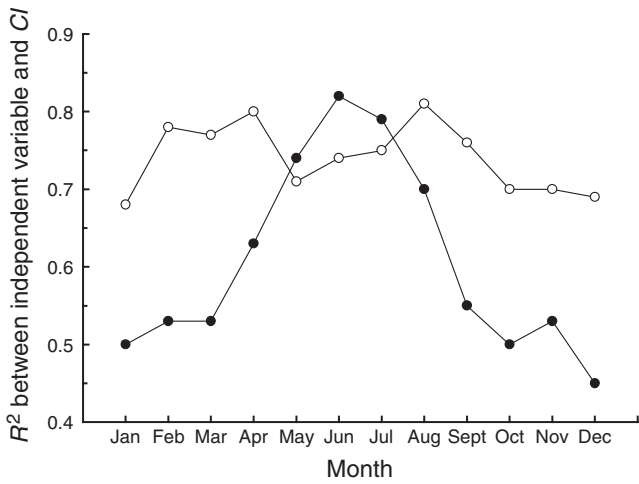


Fig. 4. Coefficient of determination (R^2) between average colonisation index (CI) (for both years) and monthly average temperature (closed circles) and monthly average minimum temperature (open circles).

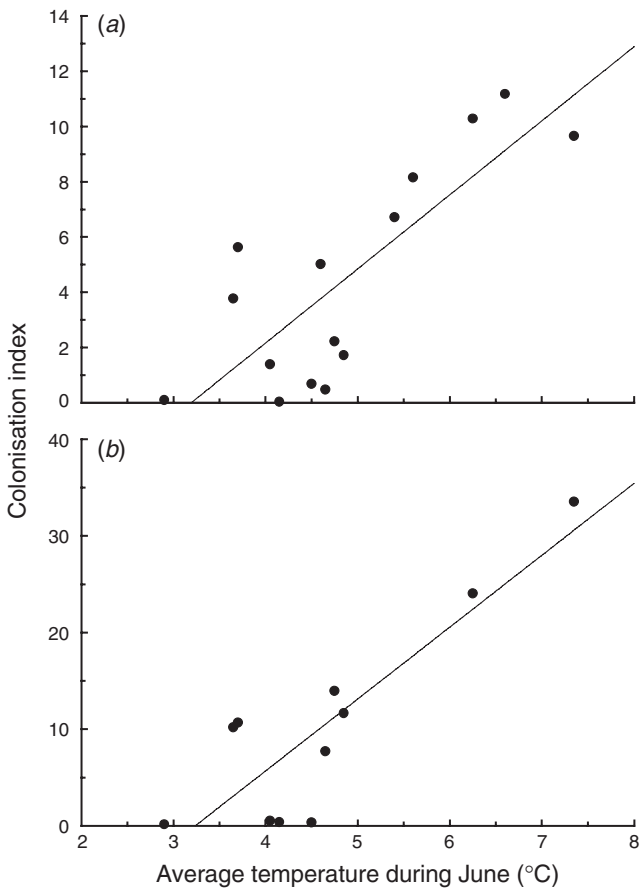


Fig. 5. Relationship between colonisation index (CI) and average temperature during June for (a) 1-year-old needles ($R^2 = 0.75$) and (b) 2-year-old needles ($R^2 = 0.75$).

needles). For both age classes of needles, plots of predicted values against actual values of CI exhibited little apparent bias (Fig. 6).

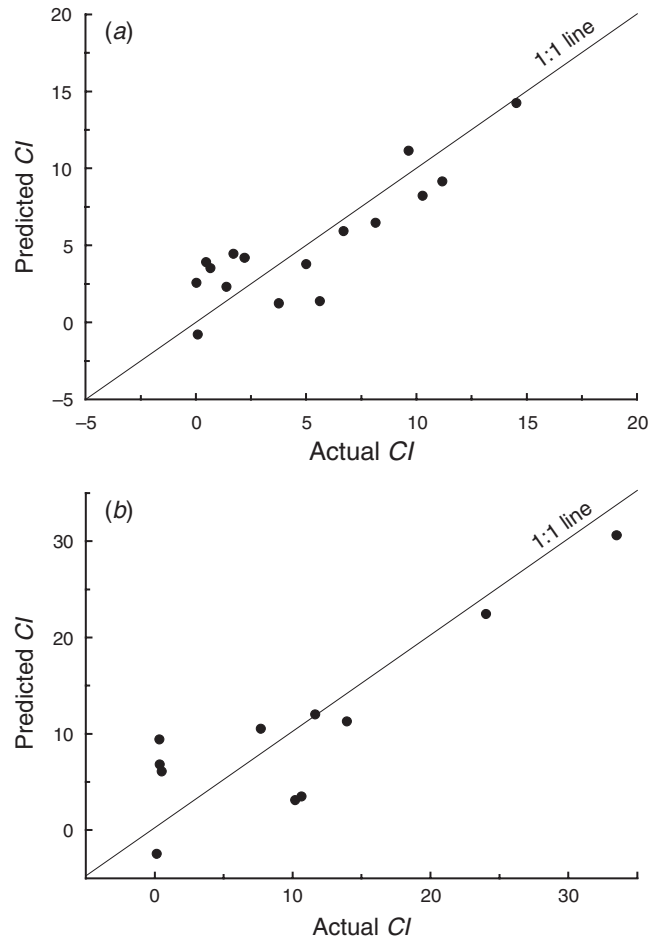


Fig. 6. Relationship between predicted and actual colonisation index (CI) for (a) 1-year-old needles and (b) 2-year-old needles. On both graphs a 1 : 1 line has been drawn.

Discussion

P. gaeumannii is widespread throughout New Zealand, but severity of Swiss needle cast disease varies with environmental conditions that influence infection and colonisation by the pathogen, as has been reported for western Oregon (Manter *et al.* 2005). Ascospores of *P. gaeumannii* are released in the early summer, and most infection occurs in current-year needles during the first few weeks of shoot emergence (Hood and Kershaw 1975; Stone *et al.* 2007). Rainfall during the infection period facilitates ascospore dispersal, and free moisture on needles provides favourable conditions for spore germination and initial infection (Stone *et al.* 2007). Patterns of precipitation during the infection period have long been recognised as a factor associated with regional variation in abundance of *P. gaeumannii* (e.g. Hood 1982; McDermott and Robinson 1989). Winter temperature also has been identified as a factor positively correlated with spatial variation in abundance of *P. gaeumannii* in western Oregon. This is presumably due to its limiting effect on the rate of colonisation of needles and development of *P. gaeumannii* following infection (Manter *et al.* 2005). Spring rainfall accounted for

less of the spatial variation in *P. gaumannii* abundance in the model than winter temperature because in western Oregon, as in much of New Zealand, spring rainfall typically occurs in abundance.

The relationships found here between average temperature during June and *CI* showed considerable similarity with those found between *CI* and average temperature during winter (December–February) by Manter *et al.* (2005) in Oregon. The relationships found between *CI* and winter temperature in New Zealand and Oregon have been plotted together in Fig. 7. Using data obtained from this study, estimates of *CI* using either average June temperatures or average winter temperatures show no discernable difference. Equations developed from Oregon data predict lower and higher values of *CI* in the 1-year-old and 2-year-old needles, respectively, at lower temperatures than the equations developed in this study. However, there is convergence for estimates from the two models in both needle age classes at higher temperatures.

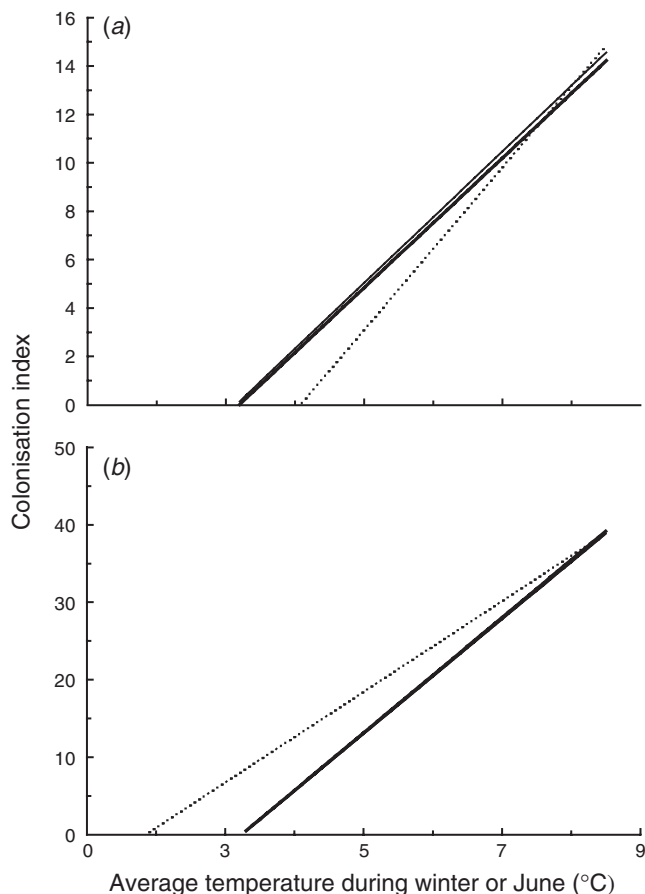


Fig. 7. Predicted colonisation index (*CI*) against average temperature during either June or winter (June–August) for (a) 1-year-old and (b) 2-year-old foliage. *CI* is shown as a function of average temperature during June (thick solid line) and average winter temperature (thin solid line), using predictive equations developed in this study. For comparison the relationship between *CI* and average temperature during winter, developed by Manter *et al.* (2005) for the Oregon Coast Range is also shown as a dotted line. In panel (b) the two solid lines are overlapping and, therefore, visible as a single line.

The spatial distribution of abundance of *P. gaumannii* and defoliation due to Swiss needle cast disease in New Zealand conforms very well with predictions based on long-term winter temperature patterns. In general, greater colonisation and less foliage retention were found in sites characterised by warmer winter temperatures in the central and eastern North Island. The Karioi site in the central North Island is a higher elevation site (about 800 m), characterised by much colder winter temperatures than the other North Island sites. This site had colonisation levels and needle retention more comparable to sites in the South Island. Winter temperature was significantly positively correlated with abundance of *P. gaumannii* for both 1- and 2-year-old foliage, as has been reported for western Oregon (Manter *et al.* 2005). To ensure that long-term averages were representative of conditions over the study period, additional analyses were undertaken using actual meteorological data. These analyses confirm results presented from long-term averages and show that the strongest environmental determinant of *P. gaumannii* was minimum temperature during June 2004 for both the 2003 ($R^2 = 0.86$; $P < 0.0001$) and 2004 ($R^2 = 0.84$; $P < 0.0001$) foliage.

Developing ascocarps of *P. gaumannii* completely block stomatal openings, thereby rendering the stoma non-functional. Occlusion of the stomata by pseudothecia of *P. gaumannii* impedes gas exchange and regulation of transpiration, causing impaired photosynthetic activity, and is considered the primary mechanism of pathogenicity (Manter *et al.* 2000, 2003). Estimates of the effect of *P. gaumannii* on CO_2 assimilation indicate that occlusion of ~25% of stomata results in overall negative needle carbon budgets, i.e. respiration exceeds assimilation, on an annual basis (Manter *et al.* 2003). Because the physiological effects of the disease (impaired CO_2 uptake and photosynthesis) are quantitatively related to the abundance of the pathogen (proportion of stomata occluded by ascocarps), ascocarp abundance is an appropriate and easily measured quantitative response variable for assessing effects of environmental factors on disease.

Incidence of infection frequently approaches 100% in 1- and 2-year old foliage where disease is moderate to severe. Therefore, *CI* was used as a response variable to provide a greater range of values for comparing *P. gaumannii* abundance. Although a maximum of 100% pseudothecial density (every stomate on a needle occluded) is theoretically possible, needles are usually shed well before 100% of stomata are occluded. Foliage abscission occurs when needles switch from being carbon sources to carbon sinks (Cannell and Morgan 1990), usually before more than half the needle stomata are occluded, because of the effect on stomatal conductance and CO_2 fixation reducing needle carbon budgets (Manter *et al.* 2003). The greatest proportion of occluded stomata observed on attached needles where disease is most severe is seldom above 50% (Hansen *et al.* 2000). The upper limit for *CI*, therefore, is also ~50.

Because the most heavily colonised needles are likely to be shed earlier than less heavily colonised needles, multiple regression relationships were examined using both the complete dataset, including all values of *CI*, and with 2003 cohort *CI* values deleted for the five sites where less than 90% of the 2003 needle cohort remained attached. The rationale for the second

analysis was that premature loss of heavily colonised needles could lead to disparities between predicted and observed values of *CI*. This was done only if the entire 2003 needle cohort was not present. Deletion of the five *CI* values did not affect variable selection, but resulted in improved fit of the combined (2003 and 2004) and 2003 cohort models.

Less foliage in the crown results in smaller annual growth increments, and reduction in volume growth (Maguire *et al.* 2002). The abundance of pseudothecia should, therefore, be correlated with the principal symptom of Swiss needle cast, premature needle abscission, because of the effect on CO₂ assimilation. Abundance of *P. gaeumannii* pseudothecia in 1- and 2-year-old needles is a measure of total disease pressure for a site (Manter *et al.* 2005), and was significantly negatively correlated with foliage retention in New Zealand. General trends of foliage retention reflected differences in *P. gaeumannii* abundance, with the greatest foliage retention occurring at South Island sites, with the exception of the Karioi site in the North Island.

Abundance of *P. gaeumannii* pseudothecia, however, only accounted for ~60% (1-year-old needles) and 57% (2-year-old needles) of the variation in needle retention across sites. The absence of a stronger relationship between *P. gaeumannii* abundance and needle retention may be attributable to several factors. First, in the case of 2-year-old foliage, the most heavily colonised needles are abscised before less heavily colonised needles in the same cohort, which could result in underestimation of disease in the sites with greater disease pressure. This may, in part, account for the smaller *R*² for 2-year-old foliage *CI* cf. needle retention. Second, considerable variation has been observed between individual trees and between tree families with respect to amount of defoliation caused by a given level of *P. gaeumannii* colonisation. Variation in needle retention under increasing disease pressure has been reported for coastal Douglas-fir provenances in New Zealand (Hood and Kimberley 2005) and the USA (Temel *et al.* 2004).

Although seed source was not found to be a significant factor explaining variation in foliage retention in this study, the *P* value for the effect of seed source, 0.08, suggests that with a wider selection of seed sources or more extensive sampling, seed source could be significantly related to foliage retention. Studies by Temel *et al.* (2003) have shown that needle retention varies across a broad sampling of coastal Douglas-fir provenances in relation to Swiss needle cast severity. Alternatively, *P* values for the relationship between seed source and *P. gaeumannii* abundance were not borderline. The effect of seed source was statistically insignificant in accounting for variation in *P. gaeumannii* colonisation. This agrees with the finding by Temel *et al.* (2004) that although heritable variation among Douglas-fir provenances and families exists as regards needle retention in the presence of *P. gaeumannii* infection, there is no detectable variation with respect to susceptibility to infection and colonisation in the coastal form of Douglas-fir (*P. menziesii* var. *menziesii*). However, others (Hood 1982; McDermott and Robinson 1989; Hood *et al.* 1990) have reported inherent differences in incidence of *P. gaeumannii* infection between interior (*P. menziesii* var. *glauca*) and coastal forms of Douglas-fir. Differences in foliage retention among coastal form Douglas-fir provenances have been interpreted

as reflecting genetic variation in disease tolerance rather than disease resistance (Temel *et al.* 2004; Hood and Kimberley 2005). Differences in disease tolerance between provenances may be undetectable under severe disease, even for seed sources that display variation in disease tolerance under moderate disease pressure (Kastner *et al.* 2001).

The potential effect of pathogen genetic variation on disease severity in New Zealand is unknown. The population of *P. gaeumannii* in western North America has been found to comprise two distinct, non-recombining, sympatric lineages, i.e. two cryptic species (Winton *et al.* 2006). Both lineages are present within the Swiss needle cast epidemic area along the northern Oregon coast, and the relative abundance of one of the lineages, currently designated Lineage 2, has been associated with more severe disease symptoms (Winton *et al.* 2006). It is unknown at this time how much variation exists in the pathogen population with respect to virulence and whether this is an important factor in epidemiology of Swiss needle cast. A preliminary sampling of *P. gaeumannii* from one location in each of the North and South Islands of New Zealand found only one of the lineages, Lineage 1. This lineage was also the only lineage found in samples from Europe and eastern North America (Winton *et al.* 2006). However, based on microsatellite allele frequencies, the North Island population grouped with a different North American population than the South Island population, suggesting at least two introduction events (L. M. Winton and J. K. Stone, unpubl. data). A more intensive sampling of *P. gaeumannii* genotypes from New Zealand is in progress to characterise the variability within the population and to determine whether multiple introductions of *P. gaeumannii* have occurred.

The results of this study suggest that variation in Swiss needle cast severity in New Zealand is being influenced by similar climate factors as those identified for the disease in western Oregon. The apparent sensitivity of *P. gaeumannii* to relatively small differences in winter temperature helps to explain patterns of spatial variation in Swiss needle cast severity, and furthermore suggests that patterns of disease distribution are being influenced by longer term climate trends. A change of +1°C average winter temperature corresponds to an increase of ~4 and 15% *CI* in 1- and 2-year-old needles, respectively, in the equilibrium winter temperature model of Manter *et al.* (2005). In comparison, the New Zealand model suggests a change of +1°C in the average temperature for June increases *CI* by 2.7 and 7.44% in 1- and 2-year-old needles, respectively. A goal of ongoing research is to refine and further test the disease prediction model to investigate interactions between climate and Swiss needle cast. An improved disease prediction model will be designed to incorporate long-term climate forecasts to enable site-specific short- and long-term disease risk predictions, growth impact predictions, and incorporate climate change models to allow examination of disease development trends under different climate scenarios. An ultimate goal is to produce a GIS-linked spatial disease prediction model for New Zealand for use as a management tool for foresters.

Acknowledgements

Funding for the project was provided by the New Zealand Foundation for Research Science and Technology and the New Zealand Douglas-

for Research Cooperative. The authors gratefully acknowledge the forest owners who authorised access to the various field sites situated within their plantations. We also thank, in particular, Judy Gardner and Wendy Sutton, for their assistance in the field and laboratory. Additional technical support was provided by Razel Blaza, Rachel Hood, Crystal Kelly, Brenda Smith, Tim Snell, Rita Tetenburg, and Travis van den Berg. We acknowledge the professional assistance of Tod Ramsfield, Darren Kriticos, Samantha Alcaraz and Mark Kimberley. Leith Knowles and Charlie Low gave advice regarding the trials belonging to the New Zealand Douglas-fir Research Cooperative. We also thank Lindsay Bulman and Luigi Gea for helpful comments on the manuscript. JKS and JLK thank Scion/Ensis for travel, logistical and financial support during the project.

References

- Beekhuis J (1978) Growth decline in Douglas-fir. In 'A review of Douglas-fir in New Zealand, FRI Symposium 15'. (Eds RN James, EH Bunn) pp. 119–125. (Forest Research Institute: Rotorua)
- Boyce JS (1940) A needle-cast of Douglas-fir associated with *Adelopus gaeumannii*. *Phytopathology* **30**, 649–659.
- Cannell MGR, Morgan J (1990) Theoretical study of variables affecting the export of assimilates from branches of *Picea*. *Tree Physiology* **6**, 257–266.
- Durrieu G (1957) Influence du climat sur la biologie de *Phaeocryptopus gaeumannii* (Rohde) Petrak, parasite du *Pseudotsuga*. *Comptes Rendus de l'Academie des Sciences Paris* **244**, 2183–2185.
- Gadgil PD (2005) 'The fungi of New Zealand. Vol. 4: fungi on trees and shrubs in New Zealand.' (Fungal Diversity Press: Hong Kong)
- Gilmour JW (1966) The pathology of forest trees in New Zealand. The fungal, bacterial, and algal pathogens. Technical paper 48. Forest Research Institute, New Zealand Forest Service. pp. 1–82.
- Hansen EM, Stone JK, Capitano BR, Rosso PH, Sutton W, Winton L, Kanaskie A, McWilliams MG (2000) Incidence and impact of Swiss needle cast in forest plantations of Douglas-fir in coastal Oregon. *Plant Disease* **84**, 773–778. doi: 10.1094/PDIS.2000.84.7.773
- Hood IA (1982) *Phaeocryptopus gaeumannii* on *Pseudotsuga menziesii* in southern British Columbia. *New Zealand Journal of Forestry Science* **12**, 415–424.
- Hood IA, Kershaw DJ (1975) Distribution and infection period of *Phaeocryptopus gaeumannii* in New Zealand. *New Zealand Journal of Forestry Science* **5**, 201–208.
- Hood IA, Kimberley MO (2005) Douglas-fir provenance susceptibility to Swiss needle cast in New Zealand. *Australasian Plant Pathology* **34**, 57–62. doi: 10.1071/AP04080
- Hood IA, Sandberg CJ, Barr CW, Holloway WA, Bradbury PM (1990) Changes in needle retention associated with the spread and establishment of *Phaeocryptopus gaeumannii* in planted Douglas-fir. *European Journal of Forest Pathology* **20**, 418–429. doi: 10.1111/j.1439-0329.1990.tb01157.x
- Hutchinson MF, Gessler PE (1994) Splines – more than just a smooth interpolator. *Geoderma* **62**, 45–67. doi: 10.1016/0016-7061(94)90027-2
- Kanaskie A, McWilliams M, Sprengel K, Overhulser D (2005) Swiss needle cast aerial surveys, 1996–2004. In 'Swiss Needle Cast Cooperative annual report 2005'. (Ed. D Shaw) pp. 9–11. (College of Forestry, Oregon State University: Corvallis, OR)
- Kastner WW, Dutton SM, Roché DM (2001) Effects of Swiss needle cast on three Douglas-fir seed sources on a low-elevation site in the northern Oregon Coast Range: results after five growing seasons. *Western Journal of Applied Forestry* **16**, 31–34.
- Knowles L, Kimberley MO, Hood IA (2001) Swiss needle cast disease of Douglas-fir – impact on growth. *Forest Health News* **114**, 1–2.
- Leathwick JR, Stephens RTT (1998) Climate surfaces for New Zealand. Landcare Research Contract Report LC9798/126. Lincoln, New Zealand.
- Maguire DA, Kanaskie A, Voelker W, Johnson R, Johnson G (2002) Growth of young Douglas-fir plantations across a gradient of Swiss needle cast severity. *Western Journal of Applied Forestry* **17**, 86–95.
- Manter DK, Bond BJ, Kavanagh KL, Rosso PH, Filip GM (2000) Pseudothecia of the Swiss needle cast fungus, *Phaeocryptopus gaeumannii*, physically block stomata of Douglas-fir, reducing CO₂ assimilation. *The New Phytologist* **148**, 481–491. doi: 10.1046/j.1469-8137.2000.00779.x
- Manter DK, Bond BJ, Kavanagh KL, Stone JK, Filip GM (2003) Modelling the impacts of the foliar pathogen, *Phaeocryptopus gaeumannii*, on Douglas-fir physiology: net canopy carbon assimilation, needle abscission and growth. *Ecological Modelling* **164**, 211–226. doi: 10.1016/S0304-3800(03)00026-7
- Manter DK, Reeser PW, Stone JK (2005) A climate based model for predicting geographic variation in Swiss needle cast severity in the Oregon Coast Range. *Phytopathology* **95**, 1256–1265. doi: 10.1094/PHYTO-95-1256
- Marks GC, Fuhrer BA, Walters NEM (1982) Swiss needle cast of Douglas-fir. In 'Tree diseases in Victoria'. (Ed. ML Huebner) pp. 44–45. Forests Commission Victoria Handbook No. 1. (Forests Commission: Melbourne)
- McDermott JM, Robinson RA (1989) Provenance variation for disease resistance in *Pseudotsuga menziesii* to the Swiss needle cast pathogen, *Phaeocryptopus gaeumannii*. *Canadian Journal of Forest Research* **19**, 244–246.
- Merkle R (1951) Über die Douglasien-Vorkommen und die Ausbreitung der Adelopus-Nadelschütte in Württemberg-Hohenzollern. *Allgemeine Forst- und Jagdzeitung* **122**, 161–191.
- Morton HL, Patton RF (1970) Swiss needle cast of Douglas-fir in the Lake States. *Plant Disease Reporter* **54**, 612–616.
- Peace TR (1962) 'Pathology of trees and shrubs.' (Oxford University Press: Oxford)
- Stone JK, Coop LB (2006) Predicting spatial variation in Swiss needle cast in western Oregon. In 'Swiss Needle Cast Cooperative annual report 2006'. (Ed. D Shaw) pp. 54–59. (College of Forestry, Oregon State University, Corvallis, OR)
- Stone JK, Capitano B, Kerrigan JL (2007) Histopathology of *Phaeocryptopus gaeumannii* on Douglas-fir needles. *Mycologia* **99**, in press.
- Temel F, Stone JK, Johnson GR (2003) First Report of Swiss Needle Cast caused by *Phaeocryptopus gaeumannii* on Douglas-fir in Turkey. *Plant Disease* **87**, 1536. doi: 10.1094/PDIS.2003.87.12.1536B
- Temel F, Johnson GR, Stone JK (2004) The relationship between Swiss needle cast symptom severity and level of *Phaeocryptopus gaeumannii* colonization in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). *Forest Pathology* **34**, 383–394. doi: 10.1111/j.1439-0329.2004.00379.x
- Winton LM, Hansen EM, Stone JK (2006) Population structure suggests reproductively isolated lineages of *Phaeocryptopus gaeumannii*. *Mycologia* **98**, 781–791.
- Winton LM, Stone JK, Hansen EM, Shoemaker RA (2007) The systematic position of *Phaeocryptopus gaeumannii*. *Mycologia* **99**, in press.

Manuscript received 14 February 2007, accepted 14 June 2007