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Dothistroma needle blight: an emerging epidemic caused by *Dothistroma septosporum* in Colombia

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Plantation forestry in Colombia is based mainly on non-native species of *Pinus* and *Eucalyptus*. Since 2008, a disease with symptoms similar to those of dothistroma needle blight (DNB) has been found affecting large areas planted to *Pinus* spp. The aim of this study was to identify the causal pathogen as well as to document the levels of disease incidence and severity. Isolates from each of three forestry zones, collected from different host species, were compared based on rDNA sequence of the ITS regions. These were conclusively identified as *Dothistroma septosporum*, one of two *Dothistroma* spp. known to cause DNB. Susceptibility was greatest on low elevation *Pinus tecunumanii* followed by *Pinus kesiya* and *Pinus oocarpa*. *Pinus maximinoi* and high elevation *P. tecunumanii* showed tolerance to *D. septosporum*. The disease incidence in the different zones varied significantly with the North zone being the most severely affected. This constitutes the first report of disease distribution and susceptibility of hosts, as well as the first consideration of the relative importance of *D. septosporum* in Colombia.

Keywords: DNA-based diagnostic, *Dothistroma septosporum*, pathogenicity, *Pinus* spp.

Introduction

Plantation forestry in Colombia is based mainly on non-native species of *Pinus* and *Eucalyptus*. Collectively, these species make up approximately 327 000 ha of plantations (MADR (Ministry of Agriculture & Rural Development), 2010) that provide the raw material for pulp and solid timber products (MADR (Ministry of Agriculture & Rural Development), 2006). Early plantations of *Pinus* spp. were largely composed of *Pinus patula*, but in recent years various other species, especially *Pinus tecunumanii* and *Pinus maximinoi*, have been planted in order to match species more appropriately to the variable sites and altitudes found in the country.

Plantations of *Pinus* spp. in Colombia have been challenged by a number of native insect defoliating pests (Vélez Angel, 1974; Wiesner & Madrigal, 1983; Mackay & Mackay, 1986; Madrigal & Abril, 1994; Rodas, 1994) and a few diseases. In 1984 a severe infection caused by *Diplodia sapinea* (syn. *Sphaeropsis sapinea*) was recorded in *P. patula* plantations in different areas in Colombia (Hoyos, 1989; Rodas & Osorio, 2008). More recently, a diverse suite of pathogens such as *Calonectria* spp. (Lombard *et al.*, 2009) and *Fusarium circinatum* (Steenkamp *et al.*, 2012) has affected *Pinus* plantations. These problems have grown in severity and this has resulted in an increasingly large area being planted with alternative, disease-tolerant species.

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Needle pathogens that have been reported in Colombia include *Lecanosticta acicola*, *Meloderma desmazierii* and *Dothistroma septosporum* (Gibson, 1979, 1980; Ivory, 1987). Gibson (1980) suggested that *M. desmazierii*, found on *Pinus radiata* and *P. patula*, would not be of any concern in pine plantations in Colombia. However, Gibson (1980) did warn that the new discovery of *L. acicola* on *Pinus elliottii*, *P. radiata* and *P. patula* in Colombia might pose a significant threat to southern hemisphere pine plantations. This was a valid concern considering the extensive disease epidemics that a similar needle pathogen, *D. septosporum*, was causing in the southern hemisphere, especially in areas such as Chile and New Zealand (Alzamora *et al.*, 2004; Bulman *et al.*, 2008).

Colombia was included in the distribution list of countries where *D. septosporum* occurs (Ivory, 1987), but there is no supporting information as to where it was found or on what host it occurred. In addition, this report was made before it was accepted that two morphologically similar species, *Dothistroma pini* and *D. septosporum*, cause the same needle blight symptoms, collectively referred to as dothistroma needle blight (DNB) or red band needle blight (Barnes *et al.*, 2004). The presence of *D. septosporum* in Colombia has thus never been unequivocally confirmed.

In 2008 a new and serious needle disease problem appeared in the central zone of Colombian pine plantations. Symptoms of the disease closely resembled those of DNB. Within 2 years, the needle blight disease had spread throughout all three forestry zones in Colombia

on the non-native *P. tecunumanii*. Importantly, this is a tree species relatively new to forestry outside its native range, and for which there is little knowledge of diseases and insect pests that affect it.

The aims of this study were to establish the distribution and host range of the new and serious needle disease in Colombia and to confirm the identity of the pathogen based on DNA sequence data. A further aim was to determine the susceptibility of different *Pinus* species and provenances of *P. tecunumanii* based on disease incidence and severity. The impact of the disease on *P. tecunumanii* in intensively managed plantations was also assessed.

Materials and methods

Disease distribution and host range

The extent of the needle blight epidemic in Colombia, mainly on *P. tecunumanii*, was assessed from field surveys conducted throughout all the plantation areas (Fig. 1) belonging to Smurfit Kappa Colombia (SKC). Initial observations commenced in 2008 and continued until 2012. The surveys covered three different geographic zones (North, Central and South) and 14 farms located in the Departments of Caldas, Risaralda, Valle del Cauca and Cauca (Fig. 1). The total area surveyed was approximately 26 730 ha and consisted of plantations of *Pinus kesiya*, *P. maximinoi*, *P. oocarpa*, *P. patula*, and low elevation (LE) and high elevation (HE) forms of *P. tecunumanii*. These two forms can be distinguished by RAPD analyses and differ slightly in morphology (Dvorak *et al.*, 2000). In their native environment in Central America, the LE form occurs between 450 and 1500 m a.s.l. and the HE form between 1500 and 2900 m a.s.l. (Dvorak *et al.*, 2000). In Colombia the optimal elevation for LE growth is between 1400 and 1900 m a.s.l.; for HE growth, the optimum is between 1900 and 2500 m a.s.l.

Pathogen identification

Sample collection, isolation and morphological characterization

To verify the identity of the pathogen responsible for the disease symptoms in the surveyed areas, infected needles bearing distinct conidiomata were collected from diseased trees in each of the three forestry zones. Needles were placed in paper envelopes and stored at 4°C in preparation for subsequent laboratory studies.

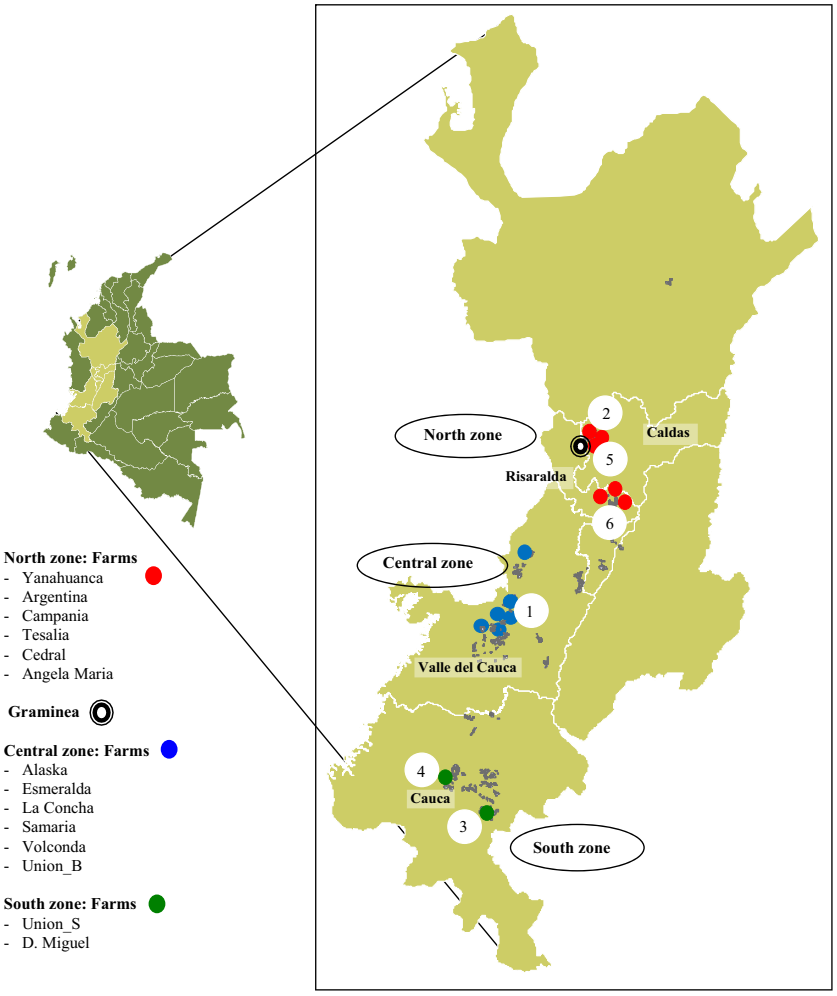

Figure 1 Geographic distribution of dothistroma needle blight (DNB) outbreaks in Colombia in the North (red dots), Central (blue dots) and South zones (green dots), on *Pinus tecunumanii*. The numbers represent the chronological order in which DNB was reported at the various locations. The black dot represents the location of the Graminea farm where the *P. tecunumanii* susceptibility trial was conducted.

Needles were prepared for isolations by first surface-disinfesting them in 0·2% sodium hypochlorite for 1 min, rinsing with distilled water and blotting them dry with sterile paper towels. Using a Nikon SMZ645 stereoscope, fruiting structures were excised from the needles and placed on malt extract agar (MEA; Merck), 1·5% (w/v) agar (Oxoid), supplemented with 1% lactic acid, and incubated for 15 days at 24°C.

Morphological characteristics of the fungus were observed using a Nikon Eclipse E200 microscope. Microscope slides were prepared by fixing conidiomata-bearing conidia, excised from diseased needles, with 1% lactic acid.

DNA sequence-based comparisons

Species identifications were made for several cultures isolated from needles collected from each of the three forestry zones and from different hosts. Mycelium was scraped from the surface of the cultures on agar, freeze-dried and crushed using the MM301 mixer mill (Retsch) for 3 min at 1/30 mHz. The crushed mycelium was heated to 65°C in 800 μL DEB buffer (200 mM Tris-HCl pH 8·0, 250 mM NaCl, 25 mM EDTA, 0·5% SDS) for 1 h and DNA was extracted using the method described by Barnes *et al.* (2001).

The rDNA internal transcribed spacer (ITS) region was amplified for the selected isolates using primers ITS1 and ITS4 (White *et al.*, 1990). The reaction mixture consisted of 5 ng DNA template, 200 nM each primer, 0·2 mM each dNTP, 1 U FastStart *Taq* DNA polymerase with 10 × buffer (Roche) and 1·5 mM $MgCl_2$. Cycling conditions were set at 96°C for 2 min; then 10 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 1 min. An additional 30 cycles were included with the annealing time altered to 40 s and a 5 s extension after each cycle with a 10 min final elongation at 72°C. PCR amplicons were visualized on 2% agarose gels and cleaned using Sephadex G-50 columns (Sigma-Aldrich). Sequencing of the amplicons was carried out using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied BioSystems) following the manufacturer's protocols and run on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Sequences were analysed in CLC BIO (MAIN WORKBENCH v. 6.6.2) and aligned using the online version of MAFFT v. 6 (http://mafft.cbrc.jp/alignment/server/). Maximum parsimony phylogenetic analyses were conducted in PAUP* v. 4.0b10 (Swofford, 2002) using the heuristic search option with random stepwise addition and tree bisection reconnection as the tree swapping algorithm. Bootstrap analyses were conducted with 1000 randomizations. The trees were rooted with sequence data for *L. acicola* as the out-group taxon.

Susceptibility of *P. tecunumanii* progenies

Susceptibility studies were established on the Graminea farm located at 5°25′19″N, 75°45′56″W, (North zone, Caldas; Fig. 1), 2155 m a.s.l., having an annual precipitation of 2560 mm and an average temperature of 18°C. At this site, 27 different *P. tecunumanii* LE progenies (half-sib crosses where the female parent is known) had been planted approximately 2 km from an area very heavily affected by needle blight. Trees in the plot were established in November 2008 and the first disease symptoms were recorded 2 years later in November 2010. A total of 1260 trees distributed evenly in six blocks were used in the trial. Each block included six trees each of 27 different *P. tecunumanii* LE progenies. For comparative purposes, each block also included six trees each of four different *P. tecunumanii* provenances [PTEBsuH1 (LE), PTEByucu (LE), PTEAcaH1 (HE), TECASaH1 (HE)], two provenances (MAXcabH1, MAXcabH2) of *P. maximinoi* and two provenances (PKcalvH1, PKcalvH2) of *P. kesiya*.

The disease incidence was assessed by dividing the total number of affected trees by the total number of planted trees for each of the *P. tecunumanii* LE progenies (972 trees representing 27 progenies) as well as for the additional treatments (288 trees representing eight provenances).

The disease severity per tree was calculated by partitioning the foliar area of the tree into equal quarters. Each quarter was then assessed individually and the relative level of disease present in that quarter of the tree was recorded as a percentage of the total crown (Fig. 2). To accommodate for the difference in conical area represented by each quarter, the data collected were then statistically reweighted so that the bottom quarter of the tree would correspond to maximum 40% of the total area of tree infected, the second quarter represented 30%, the third 20%, and the crown 10%. The weighted percentage of each quarter was then summed up to obtain the overall severity per tree.

A total of six evaluations for disease incidence and severity were performed in the Graminea trial in Nov 2010, Feb 2011, May 2011, Aug 2011, Nov 2011 and May 2012. Data used to calculate the mean percentage disease incidence and severity were analysed using descriptive statistics and data evaluation for assumptions of normality and homogenous provenance/progeny variances. Analysis of variance (ANOVA) for a completely random design (six blocks) was used to test for differences between progeny and provenances. A multiple range test was used to compare means at the 5% level (SAS PROC INSIGHT). Only the data collected in May 2012 were used for calculations of disease incidence, while the mean over all six evaluation periods was used for the severity calculations.

Disease impact in plantations

Evaluations of the impact of the disease were made at 11 forestry farms distributed in the North, Central and South zones (Table 1). Circular plots, 9·78 m radius (300 m^2), contained approximately 30 planted trees (plantation density of 3 × 3 m). These plots were randomly selected within severely diseased plantations that showed a wide range of altitudes and weather conditions such as precipitation (Table 1). A total of 90 plots, representing 1% of the total affected area (one plot per every 3 ha of affected area), were evaluated.

The impact of the disease in an area was determined by calculating the disease incidence as the total number of affected trees in each plot divided by the total number of trees in the plot. The disease severity was calculated as described above for the Graminea trial. Disease evaluations were made at least four times a year from April 2009 to January 2012 for each of the 90 plots. Abiotic factors such as precipitation, elevation and age of trees (in months) were also recorded at each farm and zone for the evaluation period. Precipitation was recorded daily throughout the evaluation period.

To determine whether the different geographic areas in Colombia influenced the presence and impact of DNB in *P. tecunumanii* plantations, data obtained from the circular plots were analysed individually per farm and per zone. Data used to calculate the mean percentage disease incidence and severity were analysed using descriptive statistics and data evaluation for assumptions of normality and homogenous variances per zone and farm. ANOVA was used to test for differences between geographic areas (zones/farms). Duncan's multiple range test was used to compare means at the 5% level (SAS PROC INSIGHT). Only the data captured in January 2012 were used to

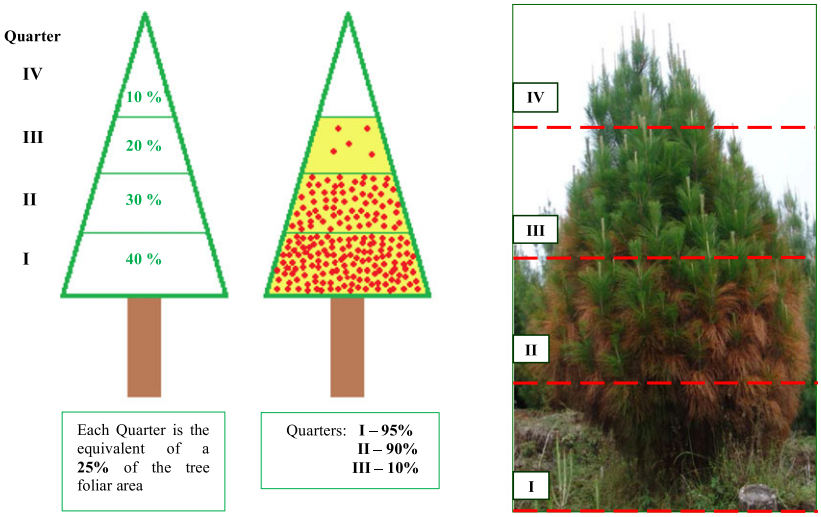

Figure 2 The disease severity of dothistroma needle blight (DNB). This is calculated by dividing the total foliar area of a tree into quarters and then estimating the percentage of infected area per each quarter. These values are then statistically analysed using a weighted mean average model based on the conical foliar area distribution of *Pinus* in which the designated value for the major quarter (bottom of the tree) corresponds to 40%, next quarter with 30%, 20%, and the top, 10%, respectively.

$$\%\,Severity = \frac{(QI * 40\%) + (QII * 30\%) + (QIII * 20\%) + (QIV * 10\%)}{100}$$

$$\%\,Severity = \frac{(95 * 40\%) + (90 * 30\%) + (10 * 20\%)}{100}$$

$$\%\,Severity = 67\%$$

Table 1 Details of the 11 forestry farms and 90 plots in Colombia used for the evaluation and impact of dothistroma needle blight on provenances of *Pinus tecunumanii*

Zone	Forestry farm	No. plots	Coordinates		m. a.s.l.	Precipitation (mm/year)[a]	Provenance planted	Date planted
			Latitude	Longitude				
North	Yanahuanca	3	5°26′12″N	75°45′46″W	2350	4389	Suiza LE[b]	Dec 2005
	Argentina	6	5°24′20″N	75°44′55″W	2244	3027	Suiza LE	Dec 2005
	Tesalia	5	4°48′15″N	75°36′31″W	1986	4082	Suiza LE	Dec 2004
		7			1908	4082	Suiza LE	Dec 2005
	Cedral	6	4°42′57″N	75°38′15″W	1902	4346	Yucul LE	Oct 2006
Central	Alaska	4	4°03′24″N	76°25′09″W	1763	2276	Yucul LE	Dec 2005
		8			1950	2276	Yucul LE	Feb 2006
	Esmeralda	4	4°03′27″N	76°25′53″W	1763	2276	Yucul LE	Dec 2005
	La Concha	1	4°00′46″N	76°25′24″W	1741	2712	Arcad LE	Jul 2004
		6			1603	2712	Yucul LE	Dec 2006
	Samaria	2	4°01′47″N	76°26′30″W	1680	2712	Suiza LE	Oct 2007
		6			1603	2712	Suiza LE	Oct 2007
		5			1745	2712	Suiza LE	May 2008
	Volconda	5	4°01′47″N	76°26′06″W	1754	2712	Yucul LE	Jun 2006
	Unión_B	5	4°25′13″N	76°15′24″W	1630	2902	Yucul LE	Aug 2006
South	Unión_S	8	2°17′04″N	76°33′56″W	2701	3102	Suiza LE	Dec 2004
		9			2780	3102	Suiza LE	Dec 2004

[a]Only the data for 2011 is presented here as an example of the amount of precipitation that can occur in 1 year.
[b]LE, low elevation.

calculate disease incidence. Severity was calculated as the mean over all evaluation periods from 2009 to 2012.

Regression analyses were used to determine whether there was a relationship between the disease severity on *P. tecunumanii* and the variables of precipitation, elevation, age (in months) of diseased trees and year of measurement (2009, 2010, 2011, 2012). Stepwise regression selects variables to include or exclude from a linear model according to the ratio of residual mean squares, which was set to 1·0 (Draper & Smith, 1981). Regression modelling was done using the statistical program GenStat (Payne, 2014).

Results

Disease distribution and host range

In June 2008 needle blight was first noticed as an important foliar disease causing significant impact on various pine species in Colombia (Fig. 3). The disease appeared in 2·5-year-old *P. tecunumanii* (Yucul LE provenance) plantations located at the Alaska and Esm-

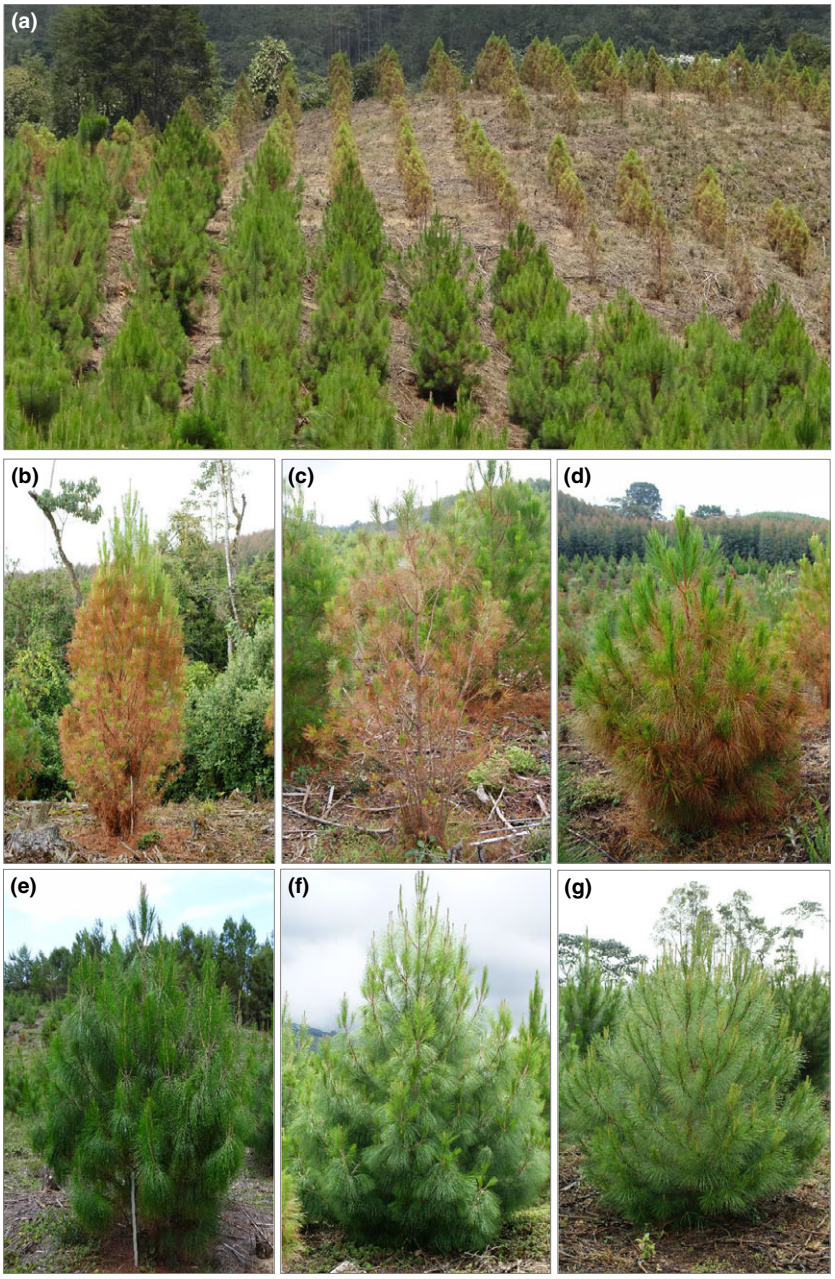

Figure 3 Dothistroma needle blight (DNB) symptoms on different pine species in Colombia. (a) Tolerant and susceptible *Pinus* species to DNB. Pine species susceptible to DNB include: (b) *P. tecunumanii* of low elevation provenance (LE), (c) *P. oocarpa*, and (d) *P. kesiya*. Species tolerant to DNB include: (e) *P. patula*, (f) *P. tecunumanii* high elevation provenance (HE), and (g) *P. maximinoi*.

eralda farms in the Valle del Cauca Department of the Central zone. A month later, a second report of the disease was made for 2·5 year-old *P. tecunumanii* (Suiza LE provenance) on the Argentina farm, in Caldas, North zone.

In February 2009 a third report of needle blight emerged from the La Unión_S farm (Cauca, South zone) in a 2·5 year-old-stand of *P. tecunumanii* (Suiza LE provenance). A fourth report was recorded in August 2009 where 2·1-year-old *P. kesiya* began to show signs of blight in the Cauca Department on both farms in the South zone (La Unión_S, and D. Miguel, 2°17′27″N, 76°39′46″W). In 2010 the fifth and sixth reports corresponded to infections on *P. oocarpa* and where species emerged as being highly susceptible to needle blight in two North zone localities, specifically the Campania (5°26′57″N, 75°45′56″W) and Angela Maria (4°49′18″N, 75°36′21″W) farms in the Caldas and Risaralda Departments, respectively (Fig. 1).

Visual observations of the needle blight disease on different species of pines distributed in all three zones showed that the most susceptible species were *P. tecunumanii* LE, followed by *P. oocarpa* and *P. kesiya* (Fig. 3). The most tolerant species were *P. patula*, followed by *P. tecunumanii* HE and *P. maximinoi* (Fig. 3).

Pathogen identification

Sample collection, isolation and morphological characterization

The early symptoms of needle blight in Colombia included yellow bands on the green needles, developing into dark-red to brown bands. In the advanced stages of the disease, infection proceeded upwards from the bases of trees and irregular-shaped acervuli emerged from the necrotic needle tissues. Hyaline, 2–5-septate, cylindrical spores, typical of *Dothistroma* spp. were observed in the excised conidiomata from infected needles.

Isolations from infected needles yielded typical callus-like *Dothistroma* cultures of various colours ranging from grey to pink. Some of the cultures produced a red exudate in the isolation medium. Six cultures were retained for further study: two from the North zone (Argentina and Tesalia), two from the Central zone (Alaska and Esmeralda), and two from the South zone (Don Miguel and Unión_S). These isolates and representative needle samples are maintained in the Mycological Herbarium at SKC, Colombia. The cultures are also maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

DNA sequence-based comparisons

The six isolates amplified using ITS primers produced amplicons of approximately 500 bp. All ITS sequences were identical and gave BLAST search results of 100% similarity to the ITS regions of *D. septosporum* available in GenBank (e.g. AY808288 from Chile and AY808289 from Ecuador). The alignment of the ITS sequences for these isolates with those of known identity and closely related species generated a data set of 455 characters. Of these, 35 characters were parsimony-informative. Phylogenetic analyses generated eight trees with a length of 161 and a consistency and retention index of 0·870 and 0·611, respectively. One representative tree is presented in Figure S1.

Susceptibility of *P. tecunumanii* progenies

Disease incidence

All 972 trees representing the 27 *P. tecunumanii* LE progenies in the Graminea trial became infected naturally with *D. septosporum* and the disease incidence ranged from 99·2 to 100% (mean 99·9%).

In stands used for comparative purposes, provenances having disease incidences of 100% included *P. tecunumanii* LE (PTEBsuH1 and PTEByucu) and *P. kesiya* (PKcalvH1 and PKcalvH2). Low disease incidence was recorded on *P. maximinoi* MAXcabH1 (8·33%) and MAXcabH2 (6·66%). High levels of tolerance to infection were recorded in *P. tecunumanii* high elevation (HE) PTEAcaH1 and TECASaH1, where no symptoms of needle blight were observed (Fig. 4).

Disease severity

Pinus tecunumanii LE progenies differed in the range of severity of damage (Table 2) from 0 to 85% (mean 39·7%). The provenances used for comparison that had similarly high levels of severity included *P. tecunumanii* LE PTEBsuH1 (39·1%), followed by *P. kesiya* PKcalvH2 (38·6%), *P. tecunumanii* LE PTEByucu (37·4%) and *P. kesiya* PKcalvH1 (28·2%). The lowest recorded disease severity was found in the provenances of *P. maximinoi* MAXcabH2 (0·76%) and MAXcabH1 (0·66%). *Pinus tecunumanii* HE provenances TECASaH1 and PTEAcaH1 showed no signs of infection (Table 2). There were significant differences between the mean disease severity observed in *P. tecunumanii* LE progenies and the provenances (MAXcabH1, MAXcabH2, PKcalvH1, PKcalvH2, PTEAcaH1, PTEBsuH1, PTEByucu, TECA-

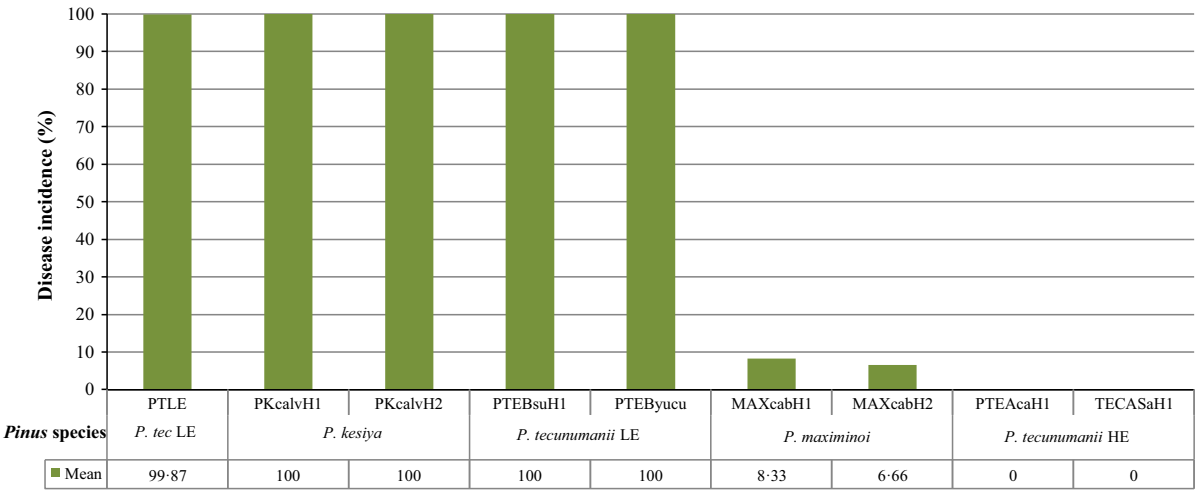

Figure 4 Disease incidence of *Dothistroma septosporum* on different progenies of *Pinus tecunumanii* LE (PTLE) and other *Pinus* species in the susceptibility trial at Graminea, North zone. LE, low elevation; HE, high elevation.

Table 2 Severity of disease caused by *Dothistroma septosporum* on *Pinus tecunumanii* and provenances of other *Pinus* species in the trial at Graminea

Pinus species	Progeny/ provenance	Disease severity (mean %)[a]	SD[b]	CV[c]	*n*[d]
P. tecunumanii LE	27 progenies	39·72 a	20·45	51·48	633
P. kesiya	PKcalvH1	28·15 b	27·20	96·63	33
	PKcalvH2	38·55 ab	26·23	68·04	29
P. tecunumanii LE	PTEBsuH1	39·10 ab	19·57	50·05	29
	PTEByucu	37·37 ab	17·54	46·49	19
P. maximinoi	MAXcabH2	0·76 c	2·33	303·89	26
	MAXcabH1	0·66 c	2·77	416·02	27
P. tecunumanii HE	PTEAcaH1	0 c	0	0	23
	TECASaH1	0 c	0	0	29

LE, low elevation provenance; HE, high elevation provenance.
[a]Duncan's multiple range test provided significance levels for the difference between pairs of means calculated between the different progenies of *P. tecunumanii* LE and provenances of other pine species. Different letters indicate significant differences at $P < 0·05$.
[b]Standard deviation.
[c]Coefficient of variation.
[d]Sample number.

Table 3 Disease severity of *Dothistroma septosporum* on *Pinus tecunumanii* calculated in plots of 300 m^2 in three forestry zones and 11 farms in Colombia

Forestry area	No. of plots	Severity (mean %)[a]
Zone		
North	27	42·38 a
Central	46	33·65 b
South	17	15·68 c
Farm		
Yanahuanca	3	37·97 b
Argentina	6	51·33 a
Tesalia	12	34·80 c
Cedral	6	50·81 a
Alaska	12	37·86 c
Esmeralda	4	34·29 c
Volconda	5	35·05 c
La Concha	7	35·98 c
Samaria	13	23·34 d
Unión_B	5	45·16 b
Unión_S	17	15·68 d

[a]Significance levels calculated for the differences in pairs of means using Duncan's mulitple range test. Different letters indicate significant differences at P < 0·05.

SaH1) used for comparative purposes ($P < 0·05$; Table 2).

Disease impact in different forestry zones

Disease incidence

The highest average disease incidence was in the North zone (98·1%, $n = 27$ plots). This was followed by the Central zone with 96·8% ($n = 46$) infection. The South zone had the lowest disease incidence, with an average of 50·6% ($n = 17$). The differences observed in the mean disease incidence between North/Central versus South zones were statistically significant ($P < 0·05$).

Disease severity

Differences in disease severity between zones and farms were statistically significant ($P < 0·05$; Table 3). *Pinus tecunumanii* in the North zone was the most severely affected by DNB with an average disease severity of 42·4% ($n = 27$) followed by the Central zone with 33·7% ($n = 46$). The lowest disease severity (15·7%; $n = 17$) was recorded in the South zone, which had approximately half the disease severity found in the Central zone (Table 3).

For the individual farms, the highest disease severity was found in Argentina (Caldas) and Cedral (Risaralda), both in the North zone with 51·3% ($n = 6$) and 50·8% ($n = 6$), respectively. The lowest disease severity levels, 23·3% ($n = 13$) and 15·7% ($n = 17$), were recorded for Samaria (Valle del Cauca, Central zone) and Unión_S (Cauca, South zone), respectively (Table 3).

In the North and Central zones, all four variables (age, elevation, precipitation and year) appeared to have an influence on disease severity (Table 4). The impact of the variables on disease severity, however, differed between zones and were not consistent. In the North, these variables contributed to 25% of the observed disease severity while in the Central zone, they accounted for 53·2%. Age of trees was related to severity in both the North and Central zones. Data for the South zone were obtained only from one farm (Union_S) and there was no indication of a relationship between disease severity and age (Table 4).

Colombia has a tropical climate and although the plantations of *P. tecunumanii* were situated at different altitudes, only a minimal level of variation in annual temperature was recorded for each zone (North = 17°C, Central = 20°C, South = 19·2°C). Temperature was considered a constant environmental factor and was, therefore, not included in the stepwise regression analyses.

In the 11 farms analysed, there was no consistent effect of age, precipitation or year on disease severity (Table 4). At La Concha the highest adjusted R^2 of 83·2% was found for a model between severity and age and year. At Volconda, age alone accounted for 62·4% of the variation in severity. Age of trees was related to severity at most farms, with the exceptions being Samaria, Union_B and Union_S (Table 4). When the age of trees in months was plotted against mean severity for each farm, no clear pattern or relationship could be determined (Fig. 5).

Discussion

Needle blight first appeared as a serious disease on pines in Colombia in 2008 where it resulted in very severe damage to various *Pinus* spp. DNA sequence comparisons for isolates from all three forestry zones, based on the rDNA ITS region, confirmed the identity of the

Table 4 Effect of elevation, precipitation and age of trees on the disease severity of *Dothistroma septosporum* on pines in different zones and at different farms in Colombia

Forestry area	Variable	Adjusted R^2	P^a	SER[b]
Zone				
North	Year	15·4	<0·001	15·10
	Elevation	19·7	<0·001	14·70
	Age	21·0	0·016	14·60
	Age.Precipitation	22·6	0·009	14·50
	Precipitation.Elevation	24·0	0·013	14·30
	Age.Elevation	25·1	0·028	14·20
Central	Age.Elevation	22·4	<0·001	12·00
	Year	25·9	<0·001	11·80
	Elevation	32·0	<0·001	11·30
	Age.Year	44·5	<0·001	10·20
	Age	51·6	<0·001	9·52
	Age.Precipitation	52·8	<0·001	9·40
	Year.Elevation	53·2	0·016	9·36
South	Age	1·0	0·138	7·93
Farm				
Yanahuanca	Age	50·2	<0·001	10·50
	Age.Year	51·3	0·187	10·30
	Year	54·5	0·079	10·00
Argentina	Age.Precipitation	32·0	<0·001	11·40
	Precipitation.Year	38·4	0·005	10·80
Tesalia	Age.Year	26·0	<0·001	12·00
	Age	28·6	0·023	11·80
	Age.Precipitation.Year	31·9	0·013	11·50
Cedral	Age	52·2	<0·001	10·80
	Age.Year	55·5	0·02	10·40
	Year	57·8	0·038	10·10
Alaska	Year	31·9	<0·001	11·00
	Age	49·7	<0·001	9·48
	Age.Precipitation	56·5	<0·001	8·81
	Precipitation.Year	59·5	0·001	8·50
Esmeralda	Age	25·2	<0·001	6·39
	Age.Year	28·7	0·08	6·25
	Year	52·0	<0·001	5·12
La Concha	Age.Year	75·8	<0·001	7·93
	Year	83·2	<0·001	6·61
Samaria	Year	4·5	0·004	7·40
	Precipitation	6·2	0·057	7·33
Volconda	Age	62·4	<0·001	9·24
Unión_B	No relationship	n/a	n/a	n/a
Unión_S	Age	1·0	0·138	7·93

[a]*P* is the probability of including a variable in the model.
[b]Standard Error of the Regression model.

pathogen as *D. septosporum*. Infection by *D. septosporum* in Colombia appears to occur throughout the year, which is different to the situation in New Zealand, for example, where the defoliation is distinctly seasonal (Bulman *et al.*, 2008). The occurrence of DNB throughout the year and without seasonal patterns in this study is consistent with the fact that high levels of precipitation are favourable for needle infection (Brown *et al.*, 2003).

The climate in large areas of Colombia where pine forestry is practised, such as those considered in this study, is conducive to DNB outbreaks. For example, the average daily temperature throughout the year for the South, Central and North zones was approximately 19, 20 and 17°C, respectively. A wide range of temperatures has been reported for *D. septosporum* infection (Gadgil, 1974), but generally long periods (48 h after inoculation) of needle wetness (Gadgil, 1974, 1977) and warmer temperatures contribute to higher disease severity. A minimum daily average temperature of 10°C and a long period of high air humidity is necessary for spore production (Dvorak *et al.*, 2012).

Precipitation was exceptionally high throughout the evaluation period and could explain why it was not shown to be a significant variable for disease severity. It is important to record that the La Niña phenomenon occurred during two consecutive years from 2009 and 2011 in Colombia and would have significantly saturated the environment with high moisture and humidity conditions. In 2011 alone, precipitation values ranged from 2276 to 4389 mm at the 11 farms evaluated. Without the effects of temperature and precipitation, the strongest variable contributing to disease severity in this study was age. This is not unexpected when it is considered that all the evaluated trees were under the age of 8 years old. Young trees are most susceptible to DNB infection (Gibson, 1972) and this is most probably why the year was also considered as an important variable at some farms. Thus, both environmental conditions and biotic factors were all highly conducive to disease development and infection by *D. septosporum* in the Colombian areas considered in this study.

An important element of this study was to confirm the identity of the pathogen associated with DNB in Colombia. Although the disease had been reported in the country previously (Gibson, 1979, 1980), this was based on incidental reports and molecular techniques were not available to confirm the identity of the pathogen. In recent years, there has been some considerable progress in refining the identity of the pathogen (Barnes *et al.*, 2004; Ioos *et al.*, 2010; McDougal *et al.*, 2011) and confirming the differences between *D. septosporum* and *D. pini*. To date, only *D. septosporum* has been found in the southern hemisphere and Central America (Groenewald *et al.*, 2007; Barnes *et al.*, 2014) and it seemed likely that this would be the species present in Colombia. Confirmation of the identity of *D. septosporum* in Colombia will now allow for comparisons to be drawn from studies on the pathogen elsewhere.

During the last approximately half century, DNB has emerged as one of the most important constraints to pine plantation forestry in the southern hemisphere. This has almost exclusively been associated with the wide-scale plantings of *P. radiata*, which is highly susceptible to the disease (Gibson *et al.*, 1964; Peterson, 1966; Gibson, 1972; Bulman *et al.*, 2008). The disease has led to the cessation of planting this tree species in many countries, notably in Africa and South America. Many new tree species have been tested and established for plantation development and it is worrying that very little is known relating to their susceptibly to pests and pathogens. The infections leading to DNB that have emerged in Colom-

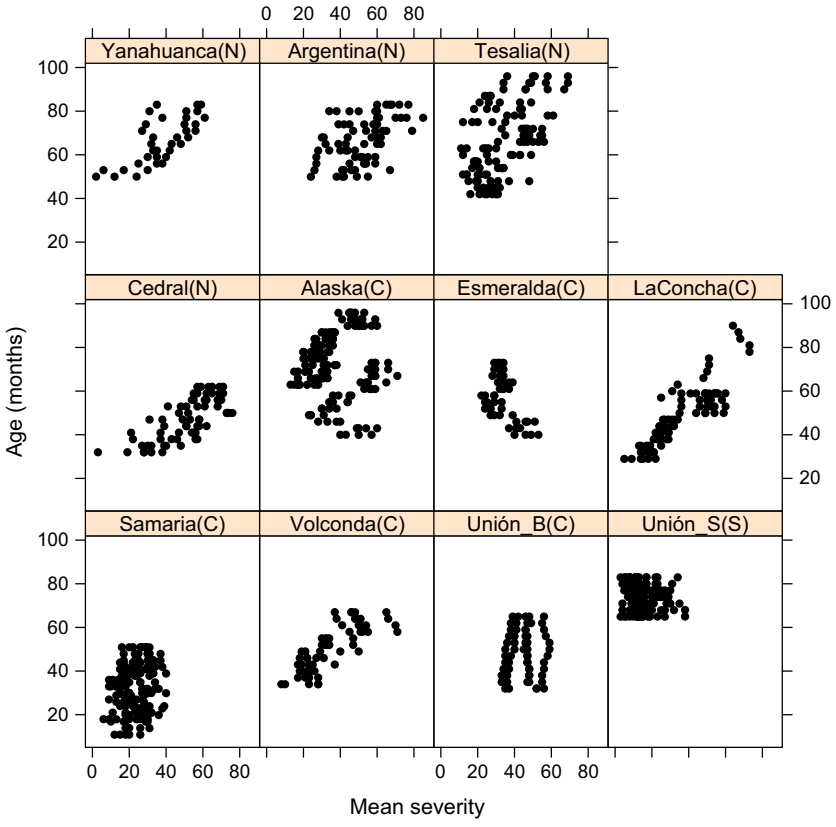

Figure 5 Comparisons between the mean severity (as a %) of *Dothistroma septosporum* infection at each of 11 farms in the North (N), Central (C) and South (S) zones with the age of pine trees at the respective farms.

bia since 2008 and reported in this study keenly reflects this situation. While DNB had been documented on *P. tecunumanii*, *P. maximinoi* and *P. kesiya* in Central America (Evans, 1984), this is the first situation where large areas of *P. tecunumanii* have been severely damaged by this, or any other serious tree pathogen (Lombard *et al.*, 2009; Steenkamp *et al.*, 2012). This is of concern because the tree is being increasingly planted outside its native range in the tropics and southern hemisphere.

The genetic diversity of the pathogen allows *D. septosporum* to adapt in new and changing environments (Dale *et al.*, 2011; Drenkhan *et al.*, 2013). Breeding programmes can be successful as long as *D. septosporum* is not able to reproduce sexually and generate genetic diversity (Hirst *et al.*, 1999). In this regard, nothing is known about the mating structure of the *D. septosporum* population in Colombia and this is a topic that requires attention. It will also be important to identify species and progenies (such as *P. tecunumanii* HE and *P. maximinoi* in this study) that have natural tolerance to *D. septosporum* in order to avoid high disease incidence in the future. This is especially important due to the increasing dispersal and impact of *D. septosporum* in native pine environments in the northern hemisphere (Drenkhan *et al.*, 2013), as well as other continents (Watt *et al.*, 2009).

Dothistroma needle blight poses a serious threat to the future of pine forestry in Colombia. This is because several provenances of *P. tecunumanii* LE, considered to be important for the future of this industry in the country, are clearly highly susceptible. In addition, other species of importance such as *P. oocarpa* and *P. kesiya* also display varying levels of susceptibility to infection. This is of concern, especially considering that planting of the previously important species *P. patula* has been substantially reduced in some areas due to the negative impact of another pathogen, *D. sapinea* (Rodas & Osorio, 2008). However, there are also good prospects to resolve the DNB problem through breeding and selection. For example, in this study some provenances of *P. tecunumanii* HE showed tolerance to DNB and could be used as a commercial species in areas that are affected by *D. septosporum* but that have optimal elevation ranges for the growth of the tree. Indeed, hybrids between tolerant genotypes of these provenances and *P. patula* already hold promise for the future (M. J. Wingfield, unpublished data).

In Colombia, disease incidence and severity was highest in the North and Central zones. During the 3-year evaluation period, and afterwards, trees in the South zone displayed substantial recovery of foliage and this contributed to low records of mean disease incidence and severity. It was not clear why the disease severity in the South zone was lower, especially as the environmental conditions and host plant species were the same. One option to resolve this question would be to investigate the population genetics of the pathogen and to determine

whether there could be a genetic explanation for the phenomenon. The durability of selected disease-tolerant planting stock would be negatively affected if both mating types and a high genetic diversity of the pathogen were present in Colombia.

Clearly a great deal of work is required to resolve the DNB problem in Colombia. In addition to breeding and selection for disease tolerance, it will be necessary to better understand the genetics of the pathogen in the country. Such studies, and others including those aimed at better understanding the biology of the pathogen in Colombia, will be important goals for the future.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Phylogenetic tree showing the placement of the isolates from Colombia in the *Dothistroma septosporum* clade.