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Report on visit to Chile to examine DFP - October 2007

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Client Report No. 16945

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Client: Forest Biosecurity Research Council
Contract No:

EXECUTIVE SUMMARY

Objective

Two Scion forest pathologists visited Chile in October 2007 to collect information on a serious new foliage disease in Chilean *Pinus radiata* plantations. The aim of the visit was to familiarise themselves with the disease and to liaise with researchers in Chile who had been working on the disease for four years.

Key Results

The disease is serious and does pose a threat to New Zealand's plantation *P. radiata* forest estate. However, it is highly likely that the disease will present a problem on sites prone to cool and wet autumn and winter conditions but will not be a problem on dry sites. The symptoms are very distinctive, but from a distance could be confused with Cyclaneusma needle cast.

Application of Results

A field guide has been written and distributed to forest health surveillance providers. Scion's diagnostic protocols for sampling diseased pine foliage have changed. The total DNA extracted in Chile will be used to test any rapid diagnostic protocols developed.

TABLE OF CONTENTS

Information for Scion abstracting:

Contract number	
Client Report No.	16945
Products investigated	
Wood species worked on	Pinus radiata
Other materials used	
Location	Chile

INTRODUCTION

Unusual symptoms on *Pinus radiata* foliage were first noted in Chile in 2003. At that time less than 100 ha were affected. By 2006, symptoms were noted over approximately 60,000 ha. In March 2006, Bioforest in Chile contacted Scion pathologists to ask their advice. At that stage the Chileans thought the blight was associated with *Diplodia pinea*, a fungus common to both countries. Chilean researchers have carried out many studies on possible causes including looking for abiotic factors such as poor nutrition, solar radiation, ozone, and soil type, as well as pests and fungal pathogens. In October 2007, Mike Wingfield from FABI in South Africa announced that an undescribed species of *Phytophthora* had been isolated from symptomatic material growing in Chile. Since then this *Phytophthora* has been described as *Phytophthora pinifolia* and pathogenicity trials have shown that *P. pinifolia* is pathogenic to *P. radiata* (Duran *et al.* 2008). The disease is known as Daño Foliar del Pino (DFP), meaning damage to pine foliage.

In October 2007, two pathologists from Scion visited Chile to look at the problem first hand. They were hosted by an official from SAG who arranged an itinerary to visit affected plantations and meet with Chilean researchers.

Findings and observations made during the visit are reported here.

ITINERARY

Rebecca Ganley and Lindsay Bulman visited Chile from 15 to 20 October 2007. Most of the time was spent visiting plantations near Concepción. The itinerary is shown below in Table 1 and Figure 1.

Date	Time	Activity
15 October		Arrival Santiago, Chile.
16 October	9:00-10:00	Meet with official from Agricultural Protection Division/SAG .
	11:45-12:45	Travel to Concepción.
	13:30-17:00	Meeting in Bioforest: Briefing on DFP in Chile (R. Ahumada and P. González).
		Lodging in Concepción.
17 October	8:00-17:00	Visit to Bosques Arauco plantations. Visit to monitoring plots of SAG.
	17:00-19:00	Travel to Temuco
		Lodging in Temuco.
18 October	8:00-16:00	Visit Forestal Valdivia plantations and SAG monitoring plots
	16:00-20:00	Travel to Concepción
19 October	8:00-18:00	Visit to the SAG Laboratorio at Chillán
	20:05-22:45	Travel Concepción – Santiago
20 October	9:00-10:00	Final meeting with SAG official and return to New Zealand
		Return to New Zealand

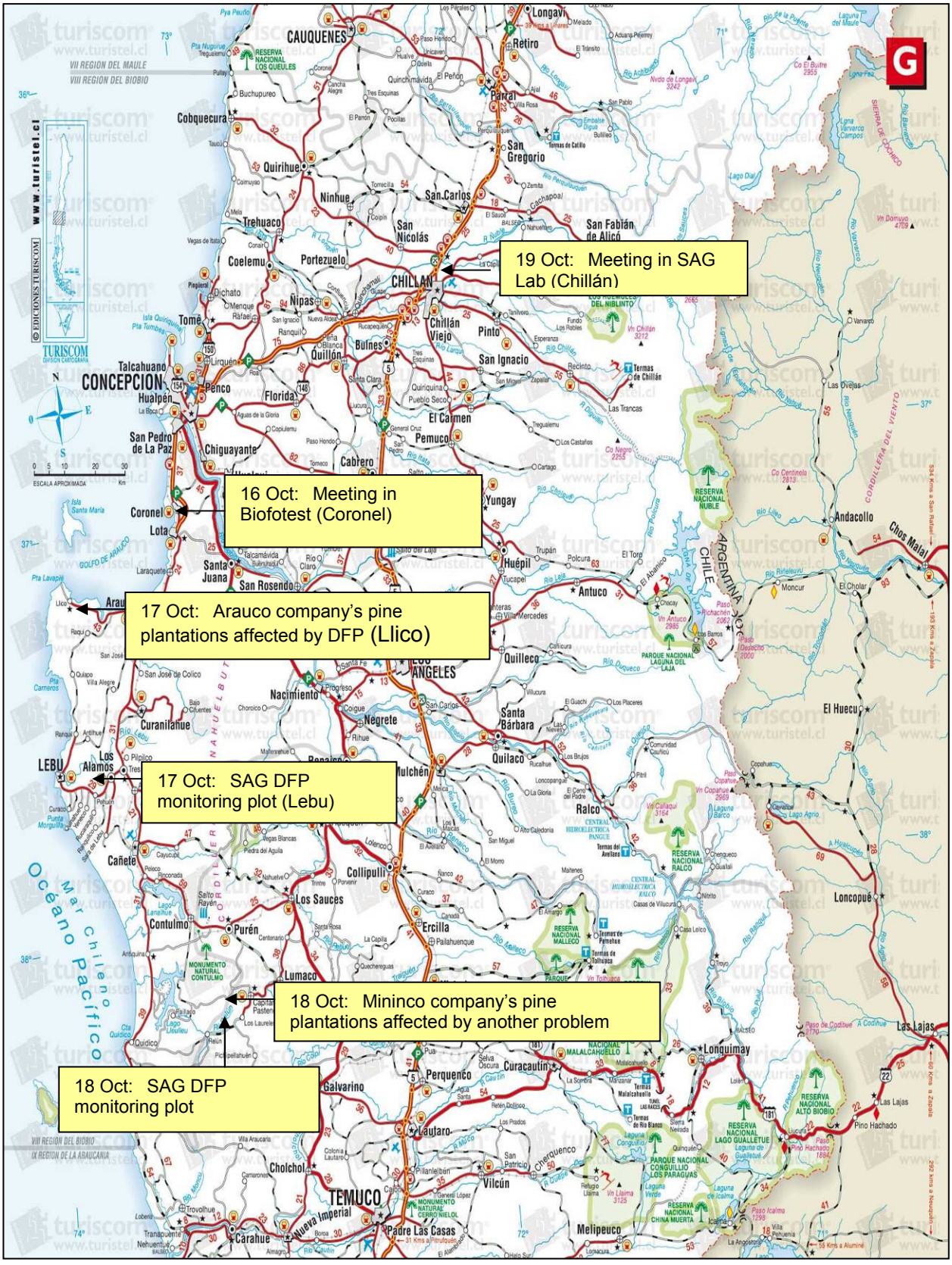


Figure 1 – Locations visited 16-19 October 2007

OBSERVATIONS

On the afternoon of 16 October a full briefing on DFP was held at Bioforest SA, Coronel south of Concepción. There were a number of Arauco employees present including Eduardo Rodriguez, Rodrigo Ahumada, and Francisco Flores. Rodriguez is the General Manager of Bioforest SA, the research branch of Arauco; Ahumada and Flores are researchers with forest pathology and tree physiology expertise, respectively. Pablo González from SAG was present along with Managers from two other forest companies. Notes were taken from a presentation by Flores and the discussion that followed.

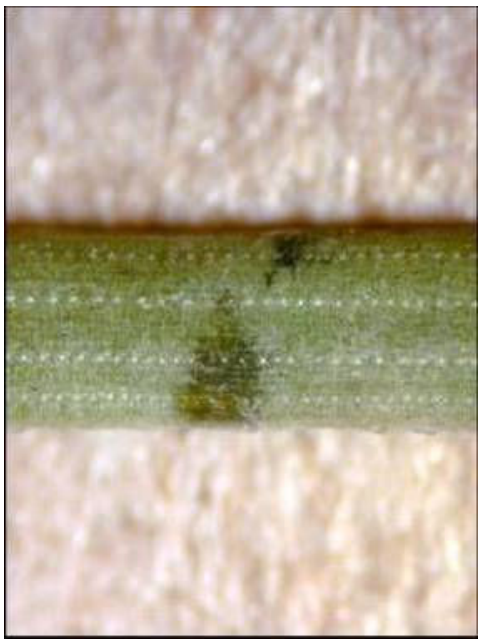
History and spread of DFP

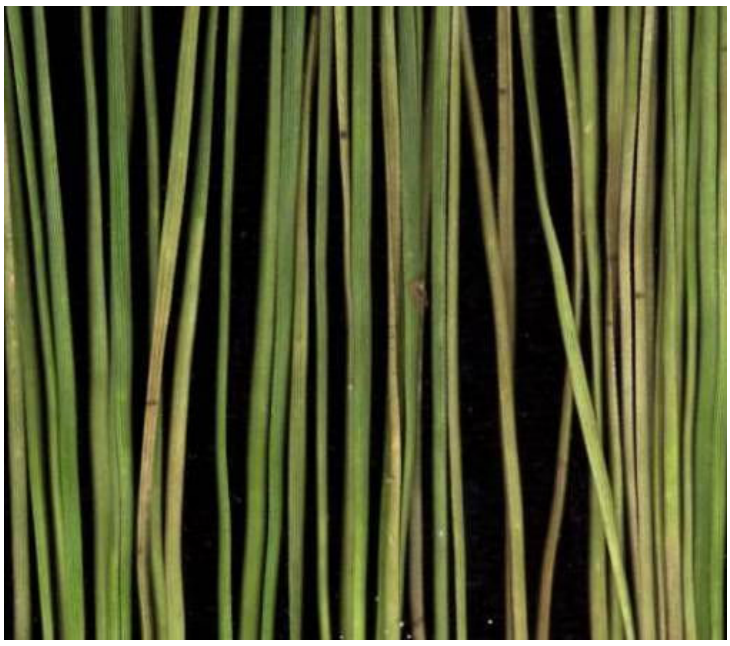
Young 6-year-old trees with unusual needle cast symptoms were first noted in 2003, over an area of 70 ha. By 2004, damage had spread throughout an area of 3,300 ha; increasing to 30,100 ha in 2005 and over 60,000 ha by 2006. The disease was first noted in Valdivia in 2006. It appears that the spread is natural rather than human. The pattern of distribution of the disease in new areas correlated with the flow of moist coastal air from north to south and then inland through valley systems.

Symptoms

In young trees, a number of symptoms are seen. Needles on the underside of branches are more commonly affected than needles on the upper side (Figure 2). The earliest symptom on needles is dark, oily-looking, bands that appear while the needle is still green. These bands usually form near the base of the needle rather than near the tip. The bands are translucent when viewed with a strongly lit background (Figure 3a). They become darker over time and finish up black (Figure 3b). As disease progresses, affected needles collapse from the base and hang down perpendicular to the branch, become yellow, and are cast. They detach readily from the branch.

Figure 2 – DFP on a young tree growing in a plantation southeast of Arauco (17 October 2007).





Figures 3a and 3b – Translucent bands associated with DFP

Figure 4 – Resin at base of needles.

Figure 5 – Lesion on a young tree.

Very young trees, 4 years old and younger, can suffer terminal wilt and dieback, particularly when growing near older infected stands.

Often resin will be seen at the point of connection between the needle fascicle and the branch (Figure 4). Sometimes brown discolouration can be found in the cambium at the trace of a dying needle. Lesions or small cankers can also form on the bark of succulent shoots on young trees (Figure 5). Lesions and cankers have not been observed on trees older than 5 or 6 years.

Older trees have similar foliar symptoms but generally do not show shoot or terminal dieback, or bark lesions and cambial discoloration. Older stands can be severely defoliated (Figure 6). Disease incidence is often extremely and unusually high where almost every tree is affected. After several years of repeated defoliation some mortality of older stands has been seen. It is thought that *Diplodia pinea* is playing a role in mortality by taking advantage of trees weakened by the repeated defoliation.

In less severely affected older stands, from a distance, symptoms appear to be similar to Cyclaneusma needle cast. Symptoms in younger trees may also be mistaken for Cyclaneusma needle cast (*see* front cover) by inexperienced observers.

Figure 6 – A severely affected 17-year-old stand in the Arauco region (taken August 2004)

Disease development and environmental factors

The time of first appearance varies. In 2006, symptoms first appeared in July. Peak symptom expression occurred in October, just before the new flush started. In 2007, symptoms were first seen in May. There was considerably less damage in 2007 compared with the previous year. The weather in autumn 2006 was considerably wetter and temperatures milder than average. In contrast, 2007 was much cooler than previous years. Wetter and cooler microsites favour DFP. Disease was strongly related to aspect where disease was worse on south-facing slopes.

It appears that the infection period is autumn/winter. Trap plant and time of planting trials showed that 100% seedling mortality occurred when planted in May through to July, 50% mortality occurred in August plantings, and no mortality was seen in September to October plantings.

Identification and diagnosis

A field identification guide describing DFP disease symptoms was prepared by Bulman and Ganley in 2007 and sent out to forest health surveillance providers. The field guide is also freely available at:
http://www.ensisjv.com/NewsEventsandPublications/ResearchInformation/ForestHealthandProtectionBulletins/tabid/314/Default.aspx
Forest health surveillance specialists and forest owners were requested to send any plants with suspicious symptoms to the Forest Health Reference Laboratory at Scion.

Protocols for testing for *P. pinifolia* were developed and documented (see Appendix 1).

Since October 2007 all *P. radiata* needle material sent to the Forest Health Reference Laboratory at Scion has been routinely tested for *P. pinifolia* according to these protocols. This includes material not displaying symptoms that resemble DFP. To date, *P. pinifolia* has not been detected in any of the material screened.

Currently there are no molecular diagnostic tests available for *P. pinifolia.* Tod Ramsfield from Scion is currently developing macroarrays for the detection of pathogens of *P. radiata*, this will include *P. pinifolia*. Total DNA extracted from needles displaying DFP symptoms in Chile was collected during the October 2007 visit and is stored at Scion. This DNA will be used to assist with the development of these molecular diagnostic tests.

CONCLUSIONS

The disease DFP is serious on some sites in Chile. There is little doubt that *P. pinifolia* is associated with the disease. Like any forest disease the severity of DFP will be governed by the interaction of host, environment, and pathogen. Accordingly, it is likely that if *P. pinifolia* established in New Zealand some *P. radiata* plantations would suffer severe damage, some would suffer intermittent damage when climatic conditions were favourable for the pathogen, and some plantations would not be affected.

ACKNOWLEDGEMENTS

Pablo González from SAG arranged the itinerary and forest visits, took us to and from the airport, and spent the week with us. We are very grateful for his enthusiastic assistance and SAG's generosity and willingness to share information with us.

REFERENCES

Duran, A; Gryzenhout, M; Slippers, B; Ahumada, R; Rotella, A; Flores, F; Wingfield, BD; Wingfield, MJ. 2008. '*Phytophthora pinifolia* sp. nov. associated with a serious needle disease of *Pinus radiata* in Chile', Plant Pathology, vol. 57, no. 4, pp. 715-727.

APPENDIX 1

Protocol for testing for Phytophthora pinifolia in pine.

Chile has a serious disease of *Pinus radiata* known as Daño Foliar del Pino (DFP), which has been found to be associated with the pathogen *Phytophthora pinifolia*. The disease is also frequently associated with both Cyclaneusma needle cast and Diplodia dieback in Chile.

What to test:

All radiata pine needle samples.

What to look for:

Needles that have unusual banding patterns/colouration, symptoms similar to Physiological needle blight (PNB)/Cyclaneusma needle cast (CNC) or have branch lesions need to be tested for *Phytophthora*.

Symptoms of DFP are most frequently dying needles which resemble what is seen with Dothistroma, Cyclaneusma, or PNB symptoms in New Zealand. Characteristic markers in the early stages of the disease are small black flecks on the needle. Later the banding is associated with resin and the infection point is transparent when you hold it up to the light. As the disease progresses the pathogen can grow into the branch and cause lesions - either resin at the base of the needle fascicle or small cankers.

Fig 1. Needle symptoms start off as black bands on the needles, often with discolouration near the fascicle. Over time this develops into what is seen in Fig 2.

Fig 2. Needle discolouration and banding, including resinous bands, associated with *Phytophthora* infection.

Fig 3. Resin associated with *Phytophthora* infection on the branch.

Fig 4. Infected tissue underneath a resin spot/infected needles.

Fig 5. Branch canker on infected pine, note the needles themselves look relatively healthy.

What to do and expect:

All putative DFP samples must have a Forest Health form and should be written up in the culture book (as with all other samples).

1. Damp chamber a branch with needles.

2. Surface sterilise and plate needle and branch samples onto SMA plates. Incubate plates at 22^{o}C for a minimum of 25 days. If any colonies grow out onto the media they will need to be tested further.

3. Bait samples with rhodo leaves – place branch and needle samples in a plastic container, cover with distilled water and float a few rhodo leaves cut in half. Leave for at least 1 week then surface sterilise the rhodo leaves, cut out any brown lesions and plate onto SMA. Plates should be incubated at 22^{o}C and left for at least one week. If anything grows these will need to be tested further.