OCCURRENCE OF *PHOMOPSIS CASTANEA* AS AN ENDOPHYTE IN CHESTNUT TREES

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ABSTRACT

Epidemiological studies on *Phomopsis* nut-rot disease of chestnuts in New Zealand revealed that the fungus *Phomopsis castanea* occurred as an endophyte in stems (except xylem), leaves, flowers and immature burrs of chestnut trees. Apparently healthy trees from nurseries, grower properties and research orchards as well as wild trees in the North and South Island were sampled and *P. castanea* was isolated from 92% of the trees. Among the four edible chestnut species and several commercial cultivars tested, the incidence was highest for trees of European chestnut (*Castanea sativa*) and cultivar 1002 and lowest in Japanese chestnut (*Castanea crenata*). The incidence of plant parts yielding *P. castanea* was also lowest for young trees from nurseries. Die-back and necrosis of male catkins and leaf spot symptoms caused by *P. castanea* were observed in some trees of *C. sativa* and American chestnut (*Castanea dentata*). Spore sampling techniques failed to recover any air-borne propagules from November 1999 to March 2000.

Keywords: *Phomopsis* nut-rot, *Castanea* spp., endophyte.

INTRODUCTION

Phomopsis nut-rot of chestnuts (*Castanea* spp.) at harvest and during storage is a serious disease in New Zealand (Klinac and Forbes 1998) causing severe economic loss to the industry. During a recent nut-quality survey, the fungus *Phomopsis castanea* was found to be associated with both rotten and healthy nuts of all cultivars sampled (Wadia *et al.* 1999) though there was a large variation in incidence between cultivars and across locations in the North and South Islands. The infection pathway for this nut-rot fungus is not well understood as there were no obvious trends for *P. castanea* to originate from either the stylar end (likely external infection) or hilum end (likely systemic infection) of the nut. Species of *Phomopsis* have been reported to be present in a latent form in the host tissue of *Fagus* and *Pinus* trees (Webber and Gibbs 1984) and also occurring as an endophyte of elm bark (Petrini and Fisher 1990). Recently, *P. castanea* was found to occur endophytically in plant parts of European chestnut (*Castanea sativa*) trees of four cultivars in Australia (Washington *et al.* 1999). The present study is part of an effort to understand the epidemiology of the disease and also identify reasons for varietal and location differences to *P. castanea* infection in New Zealand chestnuts.

METHODS

Isolations from plant material

Shoots from apparently healthy chestnut trees from nurseries, grower properties and research orchards as well as wild trees in the North (NI) and South (SI) Islands (Tables 1 and 2) were sampled between November 1999 and March 2000. From each tree, a terminal shoot (300–400 mm) comprising one year old stem, new stem, leaves,

male catkins and female flowers (or immature burrs) was collected, wrapped in newspaper and stored in a plastic bag at 4°C and processed within 2 days. Triple surface sterilised (60 s in 95% ethanol, 3 min in 2.5% NaOCl and 30 s in 95% ethanol) portions of stem separated into xylem and bark (ca 2-3 mm thick), male catkins (3 mm), leaf discs (6 mm diameter), and female flowers or small immature burrs (cut in half) were plated onto Petri plates containing potato dextrose agar (PDA, 39 g/litre) and incubated at 20°C. After 4 days of incubation, plates were observed and tissue with presence of *P. castanea* (colony recognised by a white halo with a billowing, almost fluffy white circumference) were recorded. An overall rating (see tables) was based on the percentage incidence across all plant parts yielding *P. castanea*. Data were analysed using Minitab 11, ANOVA.

Spore sampling

In an attempt to understand the dispersal mechanism of the fungus, portable high throughput 'jet' spore samplers (Burkard Manufacturing Co Ltd, UK) using a voltage of 12V-DC were operated in three orchards (two in Waikato and one at Lincoln) at different times between November 1999 and March 2000 and under various weather conditions. This spore sampler is designed for quantitative studies of living spores in an air throughput exceeding 750 litres/minute by exposing Petri plates with PDA for varying periods. Petri plates with PDA medium were also exposed to catch rain drip from canopies using hanging traps suspended on trees. About 200 Petri plates were exposed during the experimental period.

Tree symptoms

To identify the source of primary inoculum of *P. castanea*, all tree parts were thoroughly examined for visual signs of disease. Isolations were made on PDA whenever necrotic symptoms were encountered.

RESULTS

Isolations from plant material

Tables 1 and 2 show that the fungus *P. castanea* was isolated from bark, leaves, male catkins, female flowers or immature burrs of the majority of trees sampled. *P. castanea* was not isolated from xylem of any of the samples. Wild trees and cultivars of *C. sativa* had consistently higher colonisation of plant parts and rating for presence of *P. castanea* compared to other *Castanea* spp. or hybrids. Only 3 out of 56 trees sampled in the North Island and 6 out of 50 trees from the South Island did not yield *P. castanea*. Correlations between tree age (excluding isolated trees of >20 years), location (NI and SI) and rating for *P. castanea* incidence gave a highly significant ($P < 0.01$) location as well as tree age effect. However, where only cultivars growing on both North and South Islands were considered, a factorial analysis of level of infection using location and cultivars as main effects, with age as a covariate indicated only variety and location were significant. Trees from the South Island (except cultivars 1002 and Long Bay 4 from Lincoln and a *C. sativa* seedling from Karamea in the West coast) had relatively lower ratings. Old trees from Gisborne in the North Island had a lower rating (except cultivars 1001 and 1015) and *P. castanea* was mostly confined to the bark and leaves, while two 3 year old seedlings of cultivar 1718 were completely uninfected. In the Waikato region, out of 32 trees sampled, *P. castanea* was absent only in a one year old graft of cultivar 1718 and cultivar Rihei.

Spore sampling

No colonies of *P. castanea* were recovered by spore sampling from any of the locations during the entire experimental period.

Tree symptoms

Leaf spot disease started as small necrotic specks distributed all over the leaf, which later turned to irregular black spots with a chlorotic halo. The entire leaf became chlorotic with time, resulting in defoliation. Tips of male catkins showed typical die-back and sometimes, the whole catkin dried and fell off. The symptoms were observed even before the pollen had formed and necrotic parts had disrupted pollen production. The leaf spot and die-back were observed only on cultivars 2418 T (*C. dentata*) and Omo 6 (*C. sativa*) and are reported here for the first time.

TABLE 1: Incidence of endophytic *P. castanea* from chestnut trees in the North Island.

Location	Cultivar ¹	<i>Castanea</i> spp ²	Tree age (years)	Plant parts with <i>P. castanea</i> ³	Rating ⁴
Auckland	Wild seedlings (3)	C. s	40	B, L, MC, IB	4
Waikato 1 (Cambridge)	1015 Mother tree	C. c × C. s	70	B, L, MC, IB	2
	Wild seedling	C. s	20	B, L, MC, IB	4
Waikato 2 ⁵ (Rukuhia)	1002 (4)	C. c × C. s	9	B, L, MC, IB	4
	1005 (4)	C. c × C. s	9	B, L, MC, IB	2
	1015 (4)	C. c × C. s	9	B, L, MC, IB	3
	Pukekura	C. c	15	B, L, MC, IB	1
Waikato 3 ⁵ (Ohaupo)	Omo 6	C. s	10	B, L, MC, IB ⁶	4
	K 11	C. s	1	L, MC	2
	2418 M	C. d	10	MC	1
	2418 T	C. d	10	L, MC ⁶	2
	Izhizuchi	C. c	4	B, L, MC	2
	3008 B	C. m	2	B, L	1
	Rihei	C. c × C. m	4	-	0
	N 4	C. c × C. s	10	B, L, MC, IB	3
	K 19	C. c × C. s	10	B, L, MC, IB	2
	K 186	C. c × C. s	10	B, L, MC, IB	4
	K 286	C. c × C. s	10	B, L, MC, IB	4
	1718	C. d × C. s	1	-	0
	1002 (2)	C. c × C. s	6	B, L, MC, IB	4
	1005 (2)	C. c × C. s	6	L, MC	2
Waikato 4 (Tokoroa)	Wild seedling	C. s	30	B, L, MC, IB	4
	1002	C. c × C. s	8	B, L, MC, IB	4
	1015	C. c × C. s	8	B, L, MC, IB	3
Gisborne	Eastern sunrise	C. c	17	B, L	1
	1001	C. s	17	B, L, MC, IB	3
	1005	C. c × C. s	17	B	1
	1011	C. c × C. s	17	B, L	1
	1015	C. c × C. s	17	B, L, MC, IB	3
	1718	C. d × C. s	17	B, L	1
	1718 (2)	C. d × C. s	3	-	0
Manawatu 1 (Feilding)	1002	C. c × C. s	8	B, L, MC, IB	4
	1005	C. c × C. s	8	B, MC	2
Manawatu 2 (Kimbolton)	Wild seedlings (2)	C. s	20	B, L, MC, IB	4
	1002	C. c × C. s	1	B, L	2
	1005	C. c × C. s	1	B	1
	1015	C. c × C. s	1	B	1
Manawatu 3 (Palmerston North)	1002	C. c × C. s	10	B, L, MC, IB	3
	1005	C. c × C. s	10	B, L, MC, IB	4
	1007	C. c × C. s	10	B, L, MC, IB	3
	1015	C. c × C. s	10	B, L, MC, IB	3

¹Figures in parentheses indicate number of trees sampled.²C. c = *C. crenata*, C. d = *C. dentata*, C. s = *C. sativa*, C. m = *C. mollissima*.³B = bark, L = leaves, MC = male catkin, IB = immature burr.⁴Incidence of *P. castanea* from plant parts 0 = absent, 1 = < 25%, 2 = < 50%, 3 = < 75%, 4 = > 75%.⁵Spore trapping sites.⁶Trees with leaf spot and die-back of male catkin.

TABLE 2: Incidence of endophytic *P. castanea* from chestnut trees in the South Island.

Location	Cultivar ¹	<i>Castanea</i> spp ²	Tree age (years)	Plant parts with <i>P. castanea</i> ³	Rating ⁴
Lincoln ⁵	1002 (5)	C. c × C. s	9	B, L, MC, IB	4
	1005 (3)	C. c × C. s	9	B, L, MC, IB	2
	1015 (5)	C. c × C. s	9	B, L, MC, IB	2
	Herb Farm (7)	C. c × C. s	9	B, L, MC, IB	2
	Don Whelan (4)	C. s	9	B, L, MC, IB	3
	Long Bay 4 (3)	C. s	9	B, L, MC, IB	4
	Crewenna 3 (4)	C. s	9	B, L, MC, IB	2
Christchurch	Seedling	C. s	30	B, L, MC	2
	1002	C. c × C. s	4	B	1
	1005	C. c × C. s	4	L	1
	1005	C. c × C. s	7	MC	2
	1007	C. c × C. s	4	-	0
	1011	C. c × C. s	4	B	1
	1015	C. c × C. s	2	-	0
	1015	C. c × C. s	9	L	2
	Crewenna 2	C. s	3	-	0
	Crewenna 3	C. s	3	MC	1
Hanmer Springs	Seedlings (3)	C. s	50	L, MC, IB	1
Ashburton	Seedling	C. s	30	B, MC	1
West Coast	Seedling	C. s	10	B, L, MC, IB	4
Central Otago	Seedling	C. s	10	B, L, MC, IB	1
	1002	C. c × C. s	7	-	0
	1005	C. c × C. s	7	-	0
	1015	C. c × C. s	7	-	0

¹Figures in parentheses indicate number of trees sampled.

²C. c = *C. crenata*, C. d = *C. dentata*, C. s = *C. sativa*, C. m = *C. mollissima*.

³B = bark, L = leaves, MC = male catkin, IB = immature burr.

⁴Incidence of *P. castanea* from plant parts 0 = absent, 1 = < 25%, 2 = < 50%, 3 = < 75%, 4 = > 75%.

⁵Spore trapping site.

DISCUSSION

“Endophytic” and “endophyte” are positional expressions indicating that an organism is located inside plant tissue regardless of the health of the tissue (Sieber *et al.* 1990). *P. castanea* was the most predominant fungus isolated from apparently healthy trees sampled in this study. Fructifications of the fungus were not found on any part of the tree though the fungus sporulates profusely on nuts cool-stored (2–3°C) in polyethylene bags. Washington *et al.* (1999) reported higher incidence of *P. castanea* from reproductive tissue and suggested that contamination from external sources may be as important as endophytic colonisation through the peduncle. Because we so far have failed to recover any air-borne propagules, this would indicate that the nut infection is exclusively systemic. Absence of the fungus both from xylem tissue and the airdrops may have some implications for control, such as with chemical sprays, tree injections with fungicides or biocontrol agents.

Rainfall, temperature and relative humidity seem to be the most important weather factors influencing colonisation of *P. castanea* (Ogilvy 1999; Washington *et al.* 1999) and could partly explain the differences observed from year to year and between the

North and South Islands. In general, observed *Phomopsis* nut-rot has been worse in wetter, warmer regions of New Zealand, and in warm, wet years. Differences have been observed with *Phomopsis oblonga*, which colonises elm outer bark with higher frequency in the north and west compared with southern Britain (Webber and Gibbs 1984).

The presence of *P. castanea* in isolated old trees, seedlings as well as grafted trees, indicates that the fungus has been present in parts of New Zealand for a long time. At present, the three commercial cultivars 1002, 1005 and 1015 would account for nearly 95 % of the New Zealand chestnut production and all these cultivars have considerable endophytic *P. castanea* in the trees. Low levels of *P. castanea* incidence in *C. crenata*, *C. dentata* and *C. mollissima* must be further confirmed for future breeding and improved orchard management practices.

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