Phomopsis castanea infection in chestnuts from Canterbury, New Zealand

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Abstract

This paper describes a study to identify genotype and circumstance differences in the rate, levels and location of *Phomopsis castanea* (Sacc.) infection during the vegetative, flowering and early fruiting stages of chestnuts. These differences have been studied in both pollinator and main production type cultivars. This paper reports results from the orchard at Lincoln University during the 1998-1999 season. The purposes of this research were to characterise levels of in different tree lines, to partially describe the epidemiology of the disease and to identify selection parameters to achieve this goal. These data suggest that the Canterbury climate is poorly suited to *Phomopsis* as a nut disease though it is well suited to *Phomopsis* as an endophyte during winter, early spring and possibly late autumn.

***Additional key words:** epidemiology, susceptibility, selection parameters, nut diseases, endophyte*

Introduction

The chestnut is a large, edible seed produced inside a prickly case, called a burr. Unlike other nuts it is high in carbohydrates and moisture and low in oil content, so that optimal storage conditions for the nuts are more like that of fruits than other nuts (New Zealand Chestnut Council Inc., 1997) and storage rots play a large part in determining chestnut quality. Most chestnuts grown in New Zealand are of hybrid origin (McNeil, 1997). However, Oraguzie *et al.* (1998) reported that South Island selections were mostly *C. sativa*-like while North Island selections were more *C. crenata*-like.

Worldwide, there are severe limitations posed to the commercial cultivation of chestnuts by both the chestnut blight (caused by *Endothia parasitica)* and the gall wasp (New Zealand Chestnut Council Inc., 1997). New Zealand is one of the few places left in the world, which is still free of both these pests. A diverse group of other pathogens also affect chestnut (Macdonald, 1993). These include 'bark disease' (caused by *Cryphonectria parasitica* Murr.), 'ink disease', (attributed to *Phytophthora* spp.). Basidiomycetous fungi that cause root decay and powdery mildews. Numerous environmental factors may directly influence tree health, but according to Macdonald (1993), most important is the role they play in predisposing chestnut to a variety of biotic pathogens.

New Zealand has a wide range of fungal pathogens capable of affecting chestnuts including *Phomopsis, Botryosphaeria, Penicillium, Aspergillus, Alternaria, Fusarium, Botrytis, Rhizopus, Geotrichum, Pestalotia, Phoma, Trichoderma, Sclerotinia, Colletotrichum and Glomerella* species (Klinac and Forbes, 1995). The nut rot associated with *Phomopsis castanea* causes serious losses to the Australian (Washington, Stewart-Wade *and* Hood, 1999) and New Zealand (Klinac, 1996) chestnut industries. It reduces storage life, limits export and market potential, and may cause production of the undesirable mycotoxin 'phomopsin' (Klinac, 1996). A number of studies (Klinac, 1996; Washington and Stewart-Wade, 1996) have attempted to quantify the levels of infection and develop control measures by understanding the disease cycle of the causal fungus *P. castanea.* Several hundred *Phomopsis* species names appear in the literature, many of which are based solely

on host substrate (Brayford, 1990). *Phomopsis* belongs to the Deuteromycetes (asexual fungi), in the order Sphaeropsidales (asexual spores produced in pycnidia) (Agrios, 1978).

As was pointed out by Klinac and Forbes (1995), in New Zealand, there are few data available that show how much of a problem nut fungal infections are. This, they suggested, was due to the problem of pellicle adhesion in the chestnut kernel making it difficult to assess mould infections. Klinac and Forbes (1995) have reported, overseas, chestnut loss rates due to fungal infection of up to 82%, while the more typical average was between 13-25%. The same authors have reported that in New Zealand storage trials with New Zealand chestnut selections, typical losses in 1993 were between 2-44%, and in 1994 between 2-30%.

Most of the New Zealand chestnut selections examined (1002, 1005 and 1015), have shown significant levels of *Phomopsis* infection (Klinac, 1996). However, Klinac (1997) noted that some non-commercial New Zealand selections were quite resistant to fungal infection. Stewart-Wade and Washington (1995) found that in Australia there were cultivars with low incidence of *Phomopsis* and very low natural rot levels.

Phomopsis was the most common fungus isolated from inside the chestnut, but rarely from the outside, suggesting that infection occurs well before harvest (Klinac, 1996). In an Australian study (Washington and Stewart-Wade, 1996), various parts of chestnut plants (including flowers, leaves, shoots and older wood) were tested, with *Phomopsis* detected in most plant parts in all cultivars under study. In that study summer fungicide sprays gave a degree of control of rot development in nuts while post-harvest fungicide dips did not markedly reduce rots in nuts during storage. Klinac (1996) found some evidence in New Zealand for regional differences in *Phomopsis* infection. In New Zealand, in general, North Island orchards produce larger nuts, but with more internal rot and surface moulding. South Island orchards produce smaller nuts but with much less fungal infection and rots (Gardner *et al.*, 1999).

Because chestnuts in New Zealand are essentially destined for the export market, handling and storage conditions are important (McNeil and Gardner, 1995). The microflora of chestnut during storage and the postharvest treatments are critical in determining types and levels of storage rots, and eventually storage ability. In Australia, organisms isolated most often from the shell or kernel tissue were *Phomopsis, Botrytis,* unidentified yeast, *Penicillium*, *Ceuthospora*, unidentified bacteria, *Cladosporium*, *Alternaria*, *Truncatella*, *Epicoccum* and *Phoma* (Washington, Allen and Dooley, 1997). Klinac and Forbes (1995) have pointed out that the storage of chestnuts is further complicated by the genetics of chestnut itself as each nut is an out-fertilised embryo and genetically distinct from all other chestnuts.

Because the nut rot associated with *P. castanea* is a major cause of loss during storage, this study was undertaken to determine the epidemiology of this fungus in chestnut trees.

Materials and methods

During the 1998-1999 growing season an experiment was carried out to evaluate the changes in *P. castanea* infection levels in different parts of the chestnut tree from mid November to the end of April. The monitoring of *Phomopsis* infection levels was done on samples collected from 'The 1990 Lincoln University Chestnut Trial' established on a Wakanui soil located at the Horticulture Research Area, Lincoln University, Canterbury, New Zealand (latitude 43°39'S and longitude 172°28'E) (Khan, McNeil and Samad, 1998). The trial was established to test yield and quality of a number of potential commercial lines. Full details of the management for the first nine years are given in McNeil *et al* (2000). The trial was irrigated by under tree mini-sprinklers as required to maintain >50% available soil water capacity in the soil as indicated by neutron probe readings taken on a 10-day basis. Weeds were controlled under the trees by spraying with glyphosate as required. However, the weeds were shaded out to a large degree by the trees. The trees had been pruned the previous winter to remove low branches. While leaf analyses suggested no serious nutritional problems (McNeil *et al.*, 2000), it was possible that N, Ca, K and Mg may have been low. Hence in 1998 the area was surface limed at 3t/ha and fertiliser applications were increased to 250g N, 100g P, 100g K and 25g Mg per tree delivered as a winter side dressing plus an additional 100g N per tree delivered in the summer.

This trial consisted of 17 cultivars with five replications completely randomised. For the purpose of this experiment, six cultivars were sampled, three pollinator cultivars (Long Bay-4, Don Whelan and Crewenna-3) and three main crop cultivars (1005, 1015 and 1002). For each cultivar, four randomly chosen replicates (from 4 different trees) were sampled. Twigs about 30-40 cm long, with leaves and flowers were cut and put in individual plastic bags for same day laboratory processing. All samples were removed from the tree up until the final sample date when the nuts and burrs were collected from the ground.

Isolation

For each twig, four stem disks were cut at different positions along the twig. The first three stem disks were obtained from previous season's wood, while the fourth one was obtained from new season's wood.

With a 7mm diameter cork borer, two leaf disks were extracted from the old leaves (those that had formed at the start of the season) and two leaf disks from the young leaves (those that were recently fully expanded). A 3-4 mm sample from each of four male and female catkins (divided into burrs and embryo) was also obtained from each twig when available.

All sampled disks from leaves, flowers and stem were put into a nylon mesh bag and sterilised by soaking for 3min in a 2.5% sodium hypochlorite (NaOCl) solution and then rinse-washed in sterile distilled running water for 5min. For the kernel and hypocotyl, the triple sterilisation procedure was 30s in 95% ethanol, 3min in 2.5% NaOCl and 30 s in 95% ethanol using the procedure described by Johnson *et al.* (1991) and Anderson *et al.* (1997). Bark was dissected from the stem and placed on the PDA separately.

All samples were plated onto petri plates containing potato dextrose agar (PDA, 39g/litre) under sterile Laminar Flow conditions and incubated at 25°C under a 12 h light/dark regime.

Testing for *Phomopsis*

After 6 and 7 days, the plates were examined for fungal growth. For some plates the fungi provisionally identified as *Phomopsis* were purified and their identity confirmed by examination of lactophenol stained cultures under an Olympus light microscope. Fungi

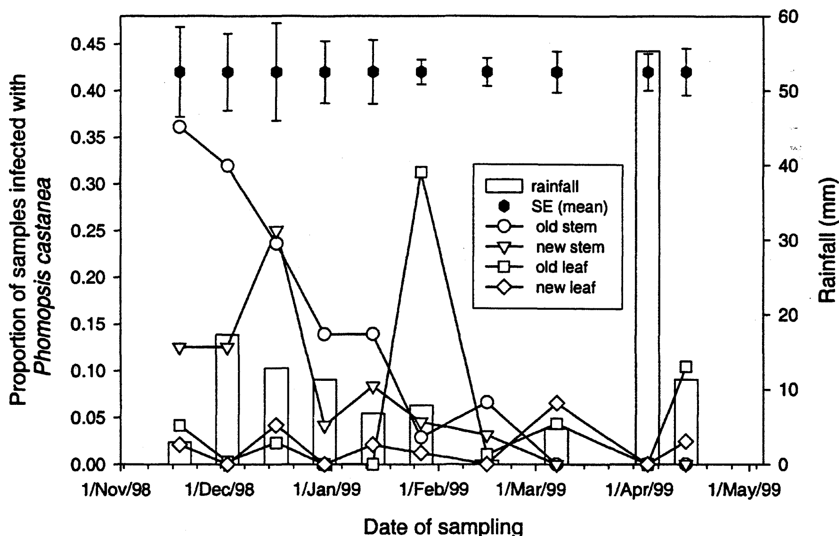

Figure 1. Proportion of vegetative samples infected *Phomopsis castanea* throughout the 1998/99 growing season. The histogram bars indicate rainfall between sample dates.

were identified on the basis of colony morphology and confirmed as *Phomopsis.*

Raw data were entered into an 'Excel' spread sheet and analysed by analysis of variance (ANOVA) using the computer package 'MINITAB-11'.

Results and Discussion

There was a general decline in the levels of "detected" infection in all chestnut plant parts sampled from the start of the season with the exception of the old leaf sample on January 27th (Fig. 1). Infection levels were low in all samples during the peak of the summer (January-February) when temperatures and solar radiation were high and rainfall low.

All cultivars examined showed *Phomopsis* infection, although these levels declined over the period of sampling up until the last few sampling dates (Fig. 2). However, there were cultivar differences in both levels of infection and in the rate of decline of infection levels (Figs. 2 and 3) with cv. Crewenna 3, Don Whelan and 1015 being particularly low. Klinac (1997) has suggested that *C. sativa* chestnut types were generally more heavily infected with fungi than *C. crenata* types. The data from this trial are not entirely consistent with this assertion as cv. Crewenna 3 and Don Whelan are very *C. sativa* like in their characteristics (Oraguzie and McNeil, 1998). However, the presence of *Phomopsis* alone could not be clearly correlated with chestnuts going rotten.

A very high level of endemic *Phomopsis* infection existed within the trial, particularly within the old bark of trees. Winter isolations from the bark have shown 100% infection of the trees tested in this trial (McNeil, pers.com. 1999). However, in spite of the high endemic levels of *Phomopsis*, the fungus may only be consistently isolated from some locations of the tree. The new growth in particular (both vegetative (Fig. 1) and reproductive (Fig. 4)) showed relatively little infestation.

There were differences in the levels of *Phomopsis* infection between the selections. This is in agreement with the observations of Klinac (1996) who found no truly *Phomopsis*-free or resistant chestnut selection but a range of levels of infection, suggesting that some

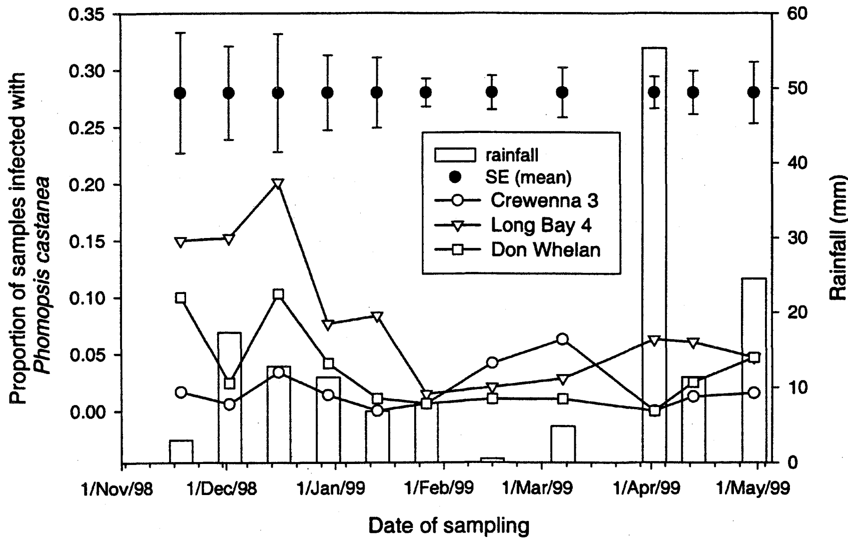

Figure 2. Proportion of samples infected with Phomopsis castanea in three pollinator varieties (Crewenna 3,Long Bay 4, and Don Whelan). Rainfall between sample dates is indicated by the histogram bars

selections may be more tolerant than others. In the present experiment, two trees were consistently *Phomopsis*-free during the entire period of the trial (tree no. 62 (cv. 1015) and 30 (cv. Crewenna-3) data not shown), but, of course, no genetic conclusion can be drawn from such observation as other trees of these cultivars were infested. In a preliminary experiment, 99% of chestnut seedlings planted out from bulk seed collected from the trial were infected with *Phomopsis*.

The observation that the bark was the main source of *Phomopsis* in samples taken from the stem was in agreement with previous results in another species, which indicated a high frequency of *Phomopsis oblonga* in elm outer bark (Webber and Gibbs, 1984).

These results must be considered as very preliminary because the weather affecting the trial area during the sampling period was unusually dry. Wetter conditions may increase the level of fungal infection (Ogilvy, 1998). Moisture, like temperature, influences the initiation and development of infectious diseases in many interrelated ways. The most important influence of moisture seems to be on the germination of fungal spores and on the penetration of the host by the germ tube (Agrios, 1978)

Previous work (Klinac, 1996) has focused mainly on the level of infection in the harvested nut, which has been found to be high (usually exceeding 50%) certainly much higher than the levels found in this trial. At this stage, there is no information on level of infection in the harvested nuts after a period of storage. However, in a study by Wadia, Klinac and McNeil (1999), it was found that the fungus *P. castanea* was associated with both rotten and healthy nuts in all the samples, though there was a large variation in severity of incidence between cultivars as well as locations. In the study, *P. castanea* was found associated with all the six cultivars from the Lincoln University orchard at very low levels. The increase in levels of infection detected in the embryos and burrs on April 13 may correspond to increased sugar levels in the maturing nut, although it is more likely related to rainfall. It has been suggested that *Phomopsis* is isolated more frequently from the hilum end of the shell and kernel than from other parts tested (Washington *et al.*, 1997). *Phomopsis castanea* has

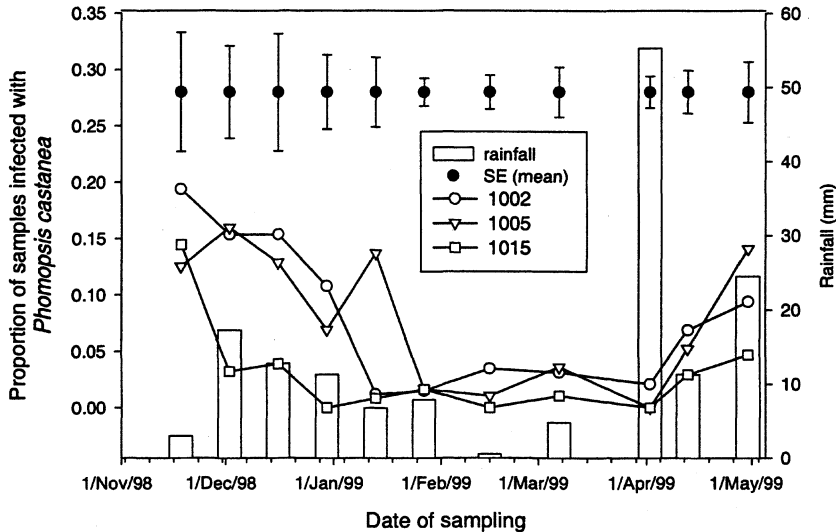

Figure 3. Proportion of samples infected with Phomosis castanea in three main crop varieties (1002,1005, and 1015). The histogram bars indicate rainfall between sample dates.

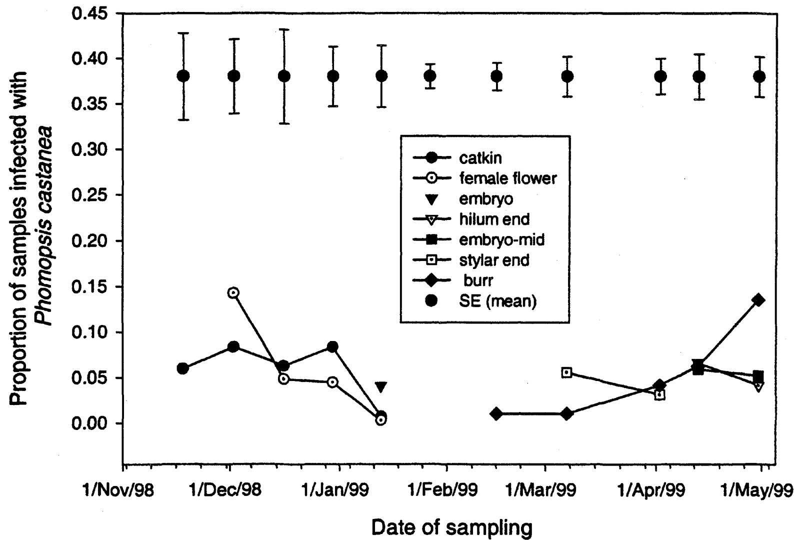

Figure 4. Proportion of chestnut samples from reproductive organs infected with Phomopsis castanea throughout the 1998/99 growing season.

been shown to be an endophyte in chestnuts (Washington, Hood, Goubran, Hepworth, Stewart-Wade, 1998) and while the mechanism of the development of the disease is not clear, it appears that the fungus colonises nuts via the peduncle and hilum (Washington *et al.*, 1998). According to Ogilvy (1998), since *Phomopsis* is an endophyte residing in the vascular system of the tree, it is unlikely that the major point of entry of the infection is via the hilum. However his observation suggests that *Castanea sativa* is more prone to infection this way than *C. crenata* or *C. mollissima.* Ogilvy (1998) believed that there is a continuing source of infection, the spores being present in most parts of the tree. However, Renhart (1999) found fungal infection (not conclusively identified as *Phomopsis*) on the stigmatic surface suggesting infection via this route rather than via the hilum. She also identified a trend for late pollinated nuts to develop a higher incidence of rot.

The data presented here do not indicate any significant difference between sample locations within the nut (Fig. 4) with infection levels in the nut always remaining low and suggesting both routes of infection are possible. The trial is being continued to determine what correlation may exist between the infection levels determined during the vegetative and flowering period and *Phomopsis*-induced rot in storage.

Conclusion

Further work in this area should involve observations over several seasons to establish the likely occurrence of genotype by environment interactions. However, at this stage, with appropriate cultivar selection, the Canterbury climate appears to be poorly suited to *Phomopsis* as a nut disease though it is well suited to *Phomopsis* as an endophyte during winter, early spring and possibly late autumn.

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