

Assessment of *Leptosphacteria polylepida* Decline in *Polylepis tarapacana* Phil. Trees in District 3 of the Sajama National Park, Bolivia

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1. Introduction

The Sajama National Park (SNP) was the first protected area (1939) in Bolivia (Fig. 1). Nowadays it is a National Park and Natural Management Area (Daza von Boeck 2005). The SNP contains a forest of the native Andean tree known as *queñua* or *quehuiña* (*Polylepis tarapacana* Phil). Forest of this type is found only in the Bolivian Andes (Argollo *et al.* 2006), where it suffers from human disturbance, including tree felling, man-made fires, the grazing of domestic animals (Toivonen *et al.* 2011) and firewood and coal extraction (Fjeldså & Kessler 2004). Indeed, its continued existence is threatened (Rivera 1998; mentioned by Daza von Boeck 2005).

The SNP occupies some 100,000 ha, with its *queñua* forest forming a belt around it (Daza von Boeck 2005). The work reported in this chapter focuses on an area on the southwestern side of District 3 of the SNP's *queñua* forest (to the northeast of the Oruro Department in the Sajama Province, on the western *Altiplano*, covering part of the Curahuara de Carangas and Turpo jurisdiction) (Fig. 1). Located between 68°40S-69°10W and 17°55S-18°15W, the SNP lies at an altitude of 4200-6600 m in the mountains below the Sajama Peak (6524 m) (Fig. 2) (Daza von Boeck 2005). The mean annual temperature in the area of the *queñua* forest is 10°C; the maximum temperature reached is 22°C, but winter temperatures can be as low as -30°C. The mean annual rainfall is 280 mm, with a range of 90-400 mm. The study area in District 3 lies at an altitude of 4200-4300 m.

2. Conservation status of the *queñua* forest

The SNP's *queñua* forest has become fragmented over centuries of human activity, leaving its animal and plant biodiversity seriously threatened (Argollo *et al.* 2006). According to

IUCN criteria, 10 of the 13 species of *Polylepis* in the Bolivian Andean region are threatened or almost threatened, the latter category being that into which *P. tarapacana* currently falls (Gareca *et al.* 2010).

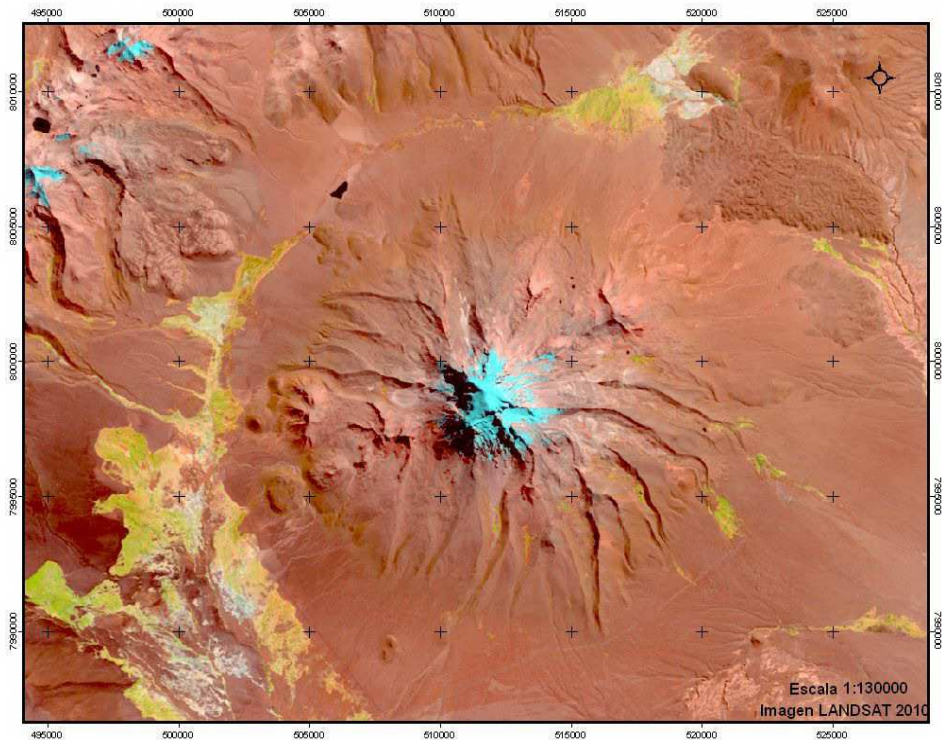


Fig. 1. Land sat satellite image TM (19/07/2011) showing the peak Sajama (light blue colour).

3. Biological factors affecting the survival of the SPN *queñua* forest

The SNP's *queñua* forest is also at risk from disease. During systematic studies of *Polylepis* in Bolivia, Kessler (pers. comm.) observed malformations of the branches - black knots similar to those formed on cherry (*Prunus* sp.) and plum trees (*Prunus domestica*). The latter author proposed that the problem might be caused by *Apiosporina morbosa* (Schwein). However, morphological and molecular analyses performed by Macía *et al.* (2005) showed *Leptosphaeria polylepidis* M.J. Macía, M. and. Palm & M.P. Martin **sp. nov.** to be the causal agent.

Leptosphaeria Ces. & Of causes different diseases in annual species. Its anamorph states are known as *Camarosporium*, *Hendersonia*, *Phoma*, *Rhabdospora* and *Stagonospora* (Hawksworth *et al.* 1995). *Leptosphaeria* species are known to affect different members of the family Rosaceae

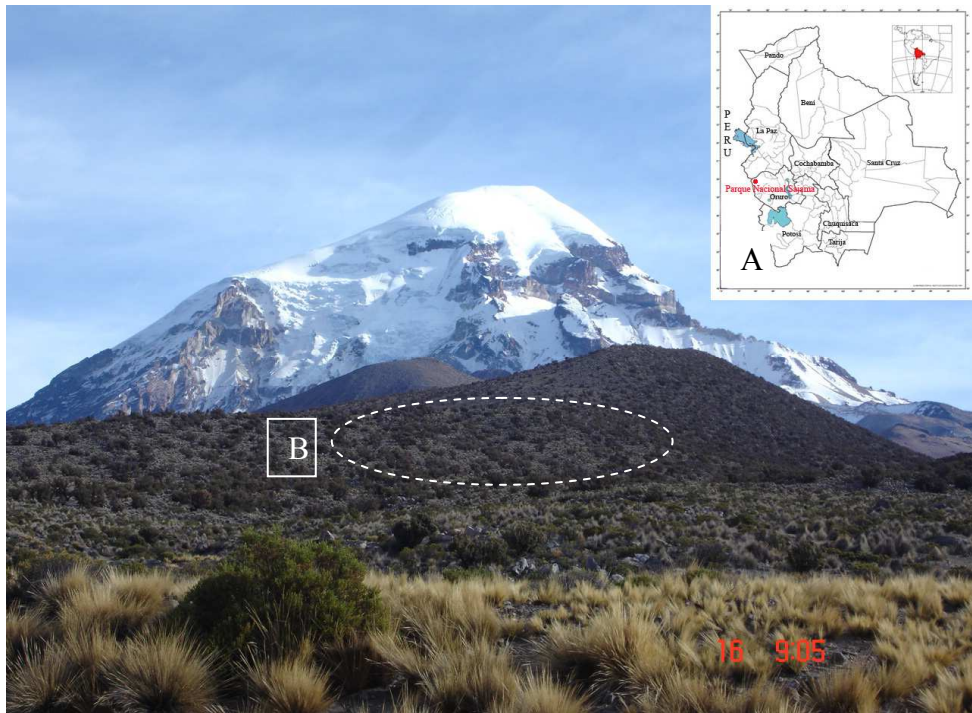


Fig. 2. South face of the snow-covered Sajama Peak (6542 m), view from the Patacamaya-Tambo Quemado international road. A: Geographical location of the Sajama National Park; B: Partial view of District 3 containing the studied *queñua* forest (white segmented line).

(Huhndorf 1992, mentioned by Macía *et al.* 2005). According to Stoykov (2004), the fungal families Phaeosporaceae and Leptosphaeriaceae (Pleosporales) are mainly saprotrophs (very rarely hemibiotrophs) that affect herbaceous stems, leaves and the floral parts of different plants.

Using transects in the southwestern sector of the SNP, beginning at a place known as Huító, Pinto Alzérreca (2007) found that of 377 trees some 35% had black knots on their branches. However, no direct relationship between the abundance of these knots and plant health was reported, nor was tree mortality found to be related to the presence of the fungus. However, in the same year, a technical report published by the SNP recorded a great increase in the mortality of *queñua* trees, particularly in District 3, naming *L. polylepidis* as the likely cause.

4. The aims of the present study were

- i. to describe the decline symptoms produced in *queñua* trees caused by *L. polylepidis*
- ii. to estimate the incidence and distribution of *L. polylepidis* decline in District 3 of the SNP's *queñua* forest.

5. Methodology

5.1 Examination of a permanent plot

A permanent plot of dimensions 100x100 m (correcting for ground undulations) was marked out in an area with representative tree density and with a structure and slope typical of District 3 of the SNP's *queñua* forest (Fig. 2). The boundaries of the plot were set using a device providing geographic positioning system (GPS) readings, a tape and compass; these boundaries ran S-N and W-E. The plot was divided into 25 segments of equal size. All *queñua* trees within the plot were labelled at a height of 1.3 m and their coordinates recorded.

5.2 Disease assessment and spatial distribution

The health of the *queñua* trees in the plot was assessed by recording the number of: *i*) apparently healthy trees (S), *ii*) diseased trees (E), *iii*) dead trees (M) and *iv*) burnt trees (Q). Trees with wilted leaves and branches and with black knots on the latter were considered diseased. The spatial distribution of the trees in each health category was determined according to Madden *et al.* (2007), with a regular pattern defined as $\sigma^2 < \mu$, a randomised pattern defined as $\sigma^2 = \mu$, and an aggregate or clustered pattern defined as $\sigma^2 > \mu$, where σ^2 = the variance of the size of the subpopulations, and μ = the mean size of the subpopulations.

5.3 Identification of the disease-causing agent

Sample pieces of branches (approximately 10-20 cm in length) were collected from: *i*) 10 trees with branches showing symptoms of decline, *ii*) 15 trees with black knots on the branches and, *iii*) 15 apparently healthy trees (Table 1). Attempts to isolate the causal agent of disease involved placing 1 cm-long branch samples in a moisture chamber at 24°C for 72 h, and culturing other 1 cm-long branch samples on two media *i*) *Queñua* Dextrose Agar (QDA) (*queñua*=250 g extract of leaves and branches), and, *ii*) Potato Dextrose Agar (PDA), according to the method of French and Hebert (1989). Fungi were identified using semi-permanent slides with lactophenol according to Macía *et al.* (2005). The anamorph state was characterised according to Sutton (1980) and Cámara *et al.* (2002).

6. Results

6.1 Symptoms and the causal agent of decline

Table 1 shows the proportion of apparently healthy trees, trees with black knots and trees with clear symptoms of decline that were positive for *L. polylepidis*.

Sample	Number of samples	Potato dextrose agar (PDA)	Queñua dextrose agar (QDA)
Apparently healthy trees	15	0%+	6%+
Trees with black knots	15	100%+	100%+
Trees with symptoms of decline	10	100%+	100%+

+ = positive for *L. polylepidis*

Table 1. Isolation of *L. polylepidis* from *P. tarapacana* samples with decline symptoms, black knots or no disease symptoms.

The decline caused by *L. polylepidis* is characterized the yellowing of the apical leaves, followed by progressive die-back of the branches from the tip downwards, by gradual defoliation of the branches and death within a few years (Fig. 3A, B, C). Slicing the bark



Fig. 3. Signs and symptoms of decline in *P. tarapacana* trees. A: partial wilting of branches; B: totally wilted dead tree (left) and apparently healthy tree (right); C: close-up showing partial wilting; D: discoloration of the vessels; E: internal view of an apparently healthy branch (upper) and of one with wilting symptoms (lower); F: stromatic bodies under the bark; G: stromatic bodies under the bark and inside the wood; H: bitunicate asci containing eight ascospores of *L. polylepidis* extracted from the inner stromatic bodies.

from partially or completely dry (dead) branches revealed a dark coloration (Fig. 3D, E), with abundant stromatic bodies visible under the ritidoma (Fig. 3F). Figure 3 G, shows black, spherical bodies incrustated in and below the bark (Fig. 3G). These spherical bodies, formed by the causal agent, contain a gelatinous mass composed of asci, ascospores and pseudo-paraphysae (Fig. 3H). These asci are bitunicate, cylindrical-clavate and contain eight ascospores with three transverse septa. The spores are brown when mature (Fig. 3H).

Most of the samples placed in the moisture chamber showed randomised ostiolate pycnial bodies distributed over the bark (Fig. 4F). These were partially immersed in the bark and



Fig. 4. Anamorphic state of *L. polylepidis* with samples showing symptoms of decline in the moisture chamber experiment. A: pieces of a branch of *P. tarapacana* showing decline symptoms; B: vascular discoloration symptoms in branches with decline; C: stromatic bodies under the ritidoma; D: samples in the moisture chamber; E: samples in the moisture chamber after 72 h of incubation at 24°C; F: close-up of stromatic bodies (red circle) and spore cloud leaving the pycnial structure of the anamorphic fungus (*Phoma* spp.) (Red colour arrows); G: Spore cloud leaving an ostiole (green arrow); the blue arrow shows the pycnial body; H: pycnial squash showing the exiting spores; I: small spores and elliptical conidia; note the almost hyaline nature of the anamorphic fungus characteristic of *Phoma* spp.

liberated their spores in a creamy-coloured cloud. The spores were small and hyaline (Fig. 4G). According to Sutton (1980), these are characteristic of *Phoma* spp. growing on QDA and PDA.

6.2 Black knots on branches and their cause

Black knots were found on the branches of both declining and apparently healthy trees (Fig. 5A). The stromatic bodies were spherical and compact (Fig. 5B, C). In cross section a thick, dark brown pseudo-parenchymatic wall was seen, with an ascus containing ascospores at

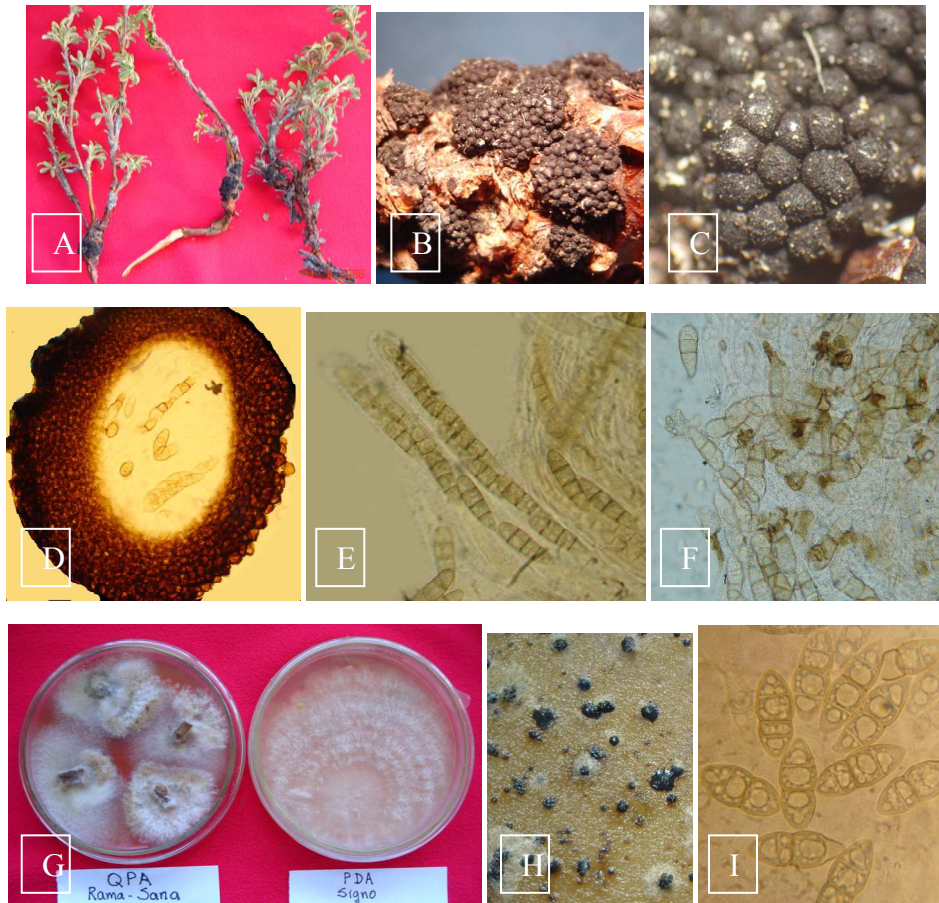


Fig. 5. A: black knots (stromatic bodies) on branches of *P. tarapacana*; B and C: close-up to the stromatic bodies; D: vertical section of conidioma showing layers with brown-melanised cells of scleropectenchyma tissue containing mature asci within; E: ascus and ascospores of *L. polylepidis*; F: ascospores of *L. polylepidis*; G: isolation of *L. polylepidis* from apparently healthy branches (left) on QDA, and from a sample with stromatic bodies on PDA; H: stromatic bodies formed on PDA after 12 days; I: ascospores extracted from these stromatic bodies.

the centre (Fig. 5D, E). Once again, the asci were typically bitunicate, cylindrical-clavate and contain eight ascospores with three clear brown septa (Fig. 5E, F). Fifteen samples of branches with black knots were all positive for *L. polylepidis* on DQA and DPA (Table 1). After three weeks on DPA, isolates from the black knots formed stromatic bodies (Fig. 5G, H). Inside these bodies gelatinous masses, formed by asci and ascospores were seen (Fig. 5I).

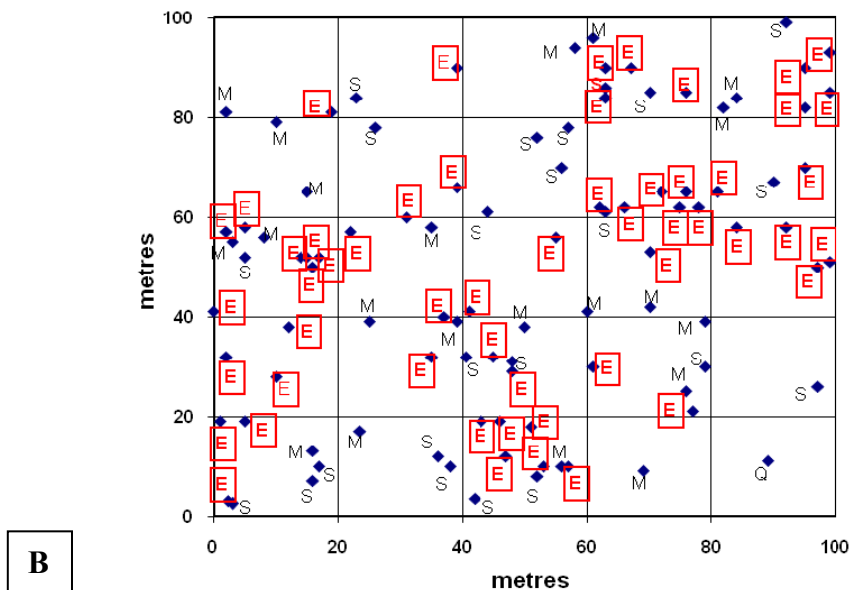
Only one of 15 apparently healthy branch samples returned a positive result for *L. polylepidis* (Table 1) (Fig. 5G). Black stromatic bodies were seen after 15 days of incubation on QDA (Fig. 5G); these contained an ascus mass and ascospores characteristic of *L. polylepidis* (Fig. 4I).

6.3 Incidence and spatial distribution

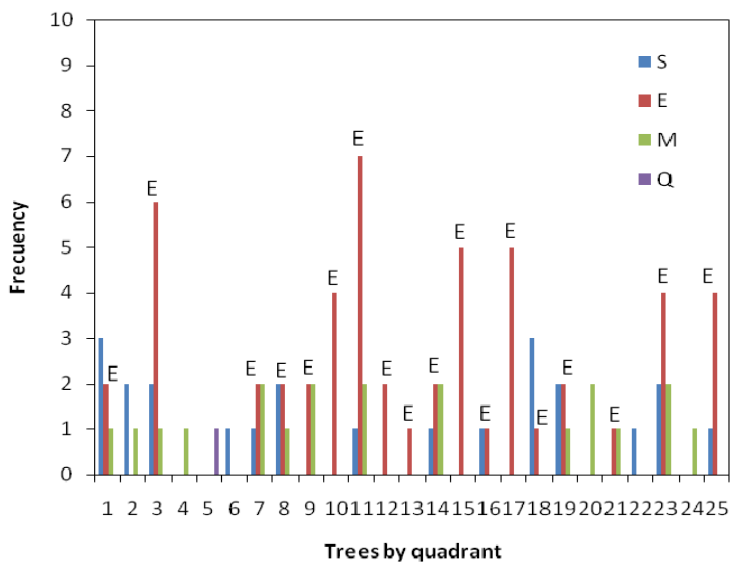
Ninety eight *queñua* trees were recorded in the experimental plot. Fifty three (54%) showed symptoms of decline (sometimes with and sometimes without black knots on the branches), twenty-one (21%) trees were dead (due to diseases and other, non-established causes), and the remaining 23 (23%) were apparently healthy (Fig. 6A). The spatial distribution of plant disease (E) in the plot showed an aggregated or clustered pattern ($\sigma^2 > \mu$ 4.3 > 2.1) (Fig. 6B). The diseased trees were distributed in 18 quadrants (72% of the total 25) and the apparently healthy trees (S) in seven (28% of the total 25) (Fig. 6C). Eleven quadrants (1, 7, 8, 9, 12, 13, 14, 16, 18, 19 and 21) had 1-2 diseased trees, and seven quadrants (3, 10, 11, 15, 17, 23 and 25) from 2-7 diseased trees (Fig. 6C).



A



B



C

Fig. 6. Incidence, spatial distribution and frequency distribution of diseased, apparently healthy, dead and burnt trees in the experimental plot. A: Incidence of disease; B: Distribution of *queñua* trees in the plot (E=diseased tree; M=dead tree; S= apparently healthy tree and Q=burnt tree); C: Frequency of healthy trees (S, blue), diseased trees (E, red), dead (M, green) and burned (Q, black) by plot quadrant.

7. Discussion

L. polylepidis is regarded as a specific pathogen of *Polylepis* spp., and was recorded on *P. tarapacana* by Macía *et al.* (2005). Coca-Morante (2008) has also recorded decline symptoms and black knots caused by *L. polylepidis* in some *P. besseri* trees growing in the Sach'aloma forest (Cochabamba, Bolivia). The climatic conditions at Sach'aloma (3800 m) are, however, totally different to those of District 3.

Decline among the trees in the studied plot was shown by wilting and/or black knots on the branches, though apparently healthy trees may also have black knots. Wilting begins apically, becoming evermore extended and intense, until the tree suffers complete defoliation and death. The formation of black knots on the branches is the only sign of *L. polylepidis* infection on living *P. tarapacana* (Macía *et al.* 2005; Pinto Alzérreca and Robledo 2006; Pinto Alzérreca 2007).

Pinto Alzérreca (2007) indicates a lack of any direct relationship between the abundance of black knots (*galls* in her terminology) and the health of the plant. This author also indicated tree mortality not to be related to the presence of fungi. However, the present results indicate that the black knots on the branches plus decline symptoms are associated with the death of *queñua* trees.

Many of the samples with symptoms of decline that were cultured in the moisture chamber showed structures of *Phoma* spp., the anamorphic state of *L. polylepidis* (Sutton 1980; Hawksworth *et al.* 1990; Cámara *et al.* 2002). The teleomorphic state of *L. polylepidis* would appear to cause monocyclic disease, while the anamorphic *Phoma* spp. state appears to be associated with polycyclic epidemics (Madden *et al.* 2007).

Black knots and symptoms of decline are usually seen in young branches. This is probably due to the ease with which the pathogen can gain access to and develop in their tissues. It is likely that the stromatic bodies formed by the pathogen under the ritidoma are related to the discoloration of the vasculature, a gradual consequence of the xylem and phloem becoming obstructed. Infection is therefore associated with the wilting seen in affected trees. According to Guest & Brown (1997), wilting results from the physical blockage of xylem vessels caused by the pathogen and, to some extent, the host response to the presence of the pathogen. Symptoms vary from yellowing, vascular browning, tylosis formation and the gumming of the vascular system, through to the general wilting of the plant. In other tree species, the vascular tissues and surrounding cortical tissues are also colonized by wilt pathogens such as the Dutch elm pathogen (*Ophiostoma ulmi*), the oak wilt pathogen (*Ceratocystis fagacearum*) and the persimmon wilt pathogen (*C. diospyri*). These pathogens are not true vascular wilt fungi, however, since their presence is not restricted to the vascular tissue. The damage caused by these agents depends largely on the extent of their cortical invasion.

The spatial distribution of disease in the plot showed an aggregated pattern. According to Madden *et al.* (2007), in aggregated patterns the points on a surface do not have an equal probability of being occupied by an individual i.e., the trees in the present plot do not have an equal probability of being infected. The dead trees (n=21) in the present work could have been killed by the studied disease but, of course, may have died of other causes. However, the detection of a single apparently healthy tree (7%) infected with *L. polylepidis* is indicative that some healthy trees are probably in the initial phases of infection. Several years may pass before symptoms become visible (generally this type of disease is associated with polyetic

epidemics) (Madden *et al.* 2007). According to the distribution of health category frequency by quadrants, it can be seen that the disease is very common in the plot area. If 7% of apparently healthy trees are infected, the disease may be having an important impact on the decline of *queñua* trees in District 3.

Decline in conjunction with black knots, at different levels of severity, was seen in 54% of the trees in the plot. However, Pinto Alzérreca (2007), who used transects in the same sector, reported 35% of 377 examined trees to show black knots. The disease therefore appears to have extended since that time. However, it is difficult to determine whether the disease is truly becoming more or less important in the SNP since no more historical data on decline symptoms or black knots are available. According to Garrett *et al.* (2009), if a disease becomes important in an area in which it was not important in the past, this may be due to changes in the climate favourable to the pathogen.

Climate change may indeed be having some effect on the *Polylepis/Lepthosphaeria* (plant/pathogen) pathosystem, and in the future may modify the spatial distribution of diseased trees. The Andean nations are likely to be among the most affected by climate change (Marengo *et al.* 2008). According to Vuille *et al.* (2003), western Bolivia can expect to experience slightly drier conditions, while Nuñez *et al.* (2008, mentioned by Marengo *et al.* 2009) suggest that northwestern Argentina and the Bolivian *Altiplano* will experience higher temperatures during the summer months and a 40% reduction in rainfall by the year 2100, leading to increasing aridity in the region. The changes experienced to date may be associated with the greater incidence of this disease.

8. Conclusion

These results strongly suggest that *L. polylepidis* is affecting *P. tarapacana* in District 3 of the SNP, causing decline and black knots on branches. Disease incidence appears to be high and to show several levels of severity. A worsening situation may be developing.

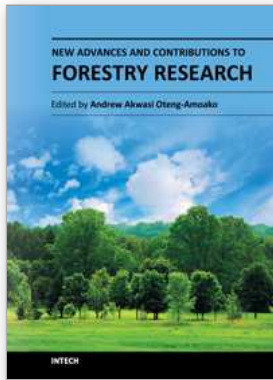
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