# AN ECTOMYCORRHIZAL ASSOCIATION WITH PROSTRATE KANUKA ON GEOTHERMAL SOILS

D. Bridge
C/o Department of Biological Sciences, University of Waikato, Hamilton.

## INTRODUCTION

Temperature and mineral abundance are two of the major features affecting the species distribution in geothermal areas. Vegetation growing on heated geothermal soil may be exposed to temperatures at the upper limit of the typical plant cell survival. These soils also have very low pH and high concentrations of potentially toxic minerals, particularly aluminium (Given, 1980; Burns, 1997; Edwards, 1998; Merrett and Burns, 1998). Increasing soil temperature leads to a decrease in species diversity, plant height and changes in plant population structure (Given, 1980; Glime and Iwatsuki, 1994; Burns, 1997). Given (1980) and Burns (1997) both observed a similar sequence of species associations along a temperature gradient at New Zealand steamfields. On cooler soils mixed forest canopy dominates and canopy height and species richness both decrease with an increase in soil temperature until low growing shrubs and mosses dominate the hotter soils (Given, 1980; Burns, 1997).

*Kunzea ericoides* var. *microflora* (prostrate kanuka) is the dominant plant species growing on the higher temperature soils at Te Kopia Scenic Reserve (Sheppard and Klyen, 1992; Burns, 1997). Given (1980) reported it surviving in 30 cm high canopies on soils of 40°C -70°C at 10cm depth. It forms low, monospecific canopies over dense bryophyte mats (Given, 1980; Ecroyd, 1991; Burns, 1997). Increases in temperatures lead to a prostrate habit and drop in density (Given, 1980; Burns and Leathwick, 1995; Burns, 1997; Merrett and Burns, 1998).

It has been suggested that the survival of geothermal plants such as prostrate kanuka is either obligatory to or facilitated by mycorrhizal associations (Burns, 1997). The presence of a mycorrhizal association increases the surface to volume ratio of the uptake system (Harley and Smith, 1983) and allows access to increased nutrient pools in depleted soils (Li *et al*, 1991). Plants infected with mycorrhizal fungi have been found to survive better on marginal lands than non-infected plants of the same species (Marx and Artman, 1979). Greater tolerance of inoculated seedlings to soil acidity, alkalinity, and ion excesses, has been demonstrated in terms of survival, size, and density (Sinclair and Marx, 1982). In addition, specific enzyme production by the fungus potentially enhances the nutrient relations, competition and pathological resistance of the fungus and its host (Duchesne *et al.*, 1988).

Prostrate kanuka is the most consistent and widespread vascular plant occurring on the heated soils of New Zealand's geothermal steamfields and sporocarps of ectomycorrhizal fungus *Pisolithus tinctorius* (deadman's foot) are commonly found near these plants (Cunningham, 1942; Burns, 1997). This is suggestive of an ectomycorrhizal association; however it has not been followed up with any structural or anatomical study. Nor had any fungal infection work been reported for *P. tinctorius* and any potential New Zealand native plant hosts. Isolation and re-infection experiments were successful in confirming the association between prostrate kanuka and *P tinctorius* and provided the necessary start for this investigation (Bridge, 2001).

*Pisolithus tinctorius* is associated with forest trees in over 30 countries where it inhabits mineral soils and marginal lands of temperate and tropical regions. It is also widely used in the inoculation and growth of plantation forestry (Marx, 1977). The ectomycorrhizal associations of *P. tinctorius* form on the terminal feeder roots of a wide range of host plant species that are a distinctive bright yellow (Brougher *et al*, 1996; Cunningham, 1944; Miller, 1982).

The role of *P. tinctorius* in sustaining and enhancing growth of conifers and eucalypts on infertile mineral soils has been well documented in the USA and Australia (Berry, 1982; Gardner and Malajczuk, 1988). It has exceptional tolerance to high temperatures in non-geothermal soils; it can grow at temperatures of 40-42°C and tolerate a wide pH range, 2.6-8.4 (Marx and Kenney, 1982). *P. tinctorius* has been found to benefit the growth of host plants in both experimental and field conditions, with infected *Pinus elliottii* grown in aseptic culture having a 25 - 180% increase in foliar weight over the controls (Marx and Bryan, 1971). Similar results have been reported in pot experiments, for example, Brougher and Malajczuk (1990) found *P. tinctorius* increased the growth of container grown *Eucalyptus diversicolor* seedlings by 50%. *Pinus* species growing on acid coal spoils with high concentrations of aluminium and manganese have been shown by Berry (1982) to have enhanced growth over 500 percent and significantly lower concentrations of toxic minerals in their shoots when *P. tinctorius* is the mycorrhizal associate. It is believed that the formation of *P. tinctorius* ectomycorrhiza at Te Kopia will have similar benefit to *K. ericoides* var. *microflora* (Burns, 1997).

The aim of the present study is to determine the response of *P. tinctorius* and its host to the extreme soil conditions at Te Kopia geothermal steamfield. This investigation was largely confined to soils with temperatures above that of 25ºC and focused on the relationship between the soil temperature, pH and the growth and distribution of *K. ericoides* var. *microflora* and the level of ectomycorrhizal association with *P. tinctorius*.

## METHODS

The Te Kopia geothermal steamfield, which is possibly the least disturbed geothermal field in the North Island (Burns, 1997), is located within the Department of Conservation administered Te Kopia Scenic Reserve, 20 kilometres south of Rotorua. It covers 95 hectares of the scarp at the base of the Paeroa fault line, ranging from the foothills to the ridge over steep terrain. Native and plantation forests as well as pastoral farmland surround this area and the soils consist of sandy silt Taupo hill soils that are very low in organic content.

Sampling was carried out at the base and foothills of the scarp where *K. ericoides* var. *microflora* forms a discontinuous canopy. Four transects were conducted. An initial one metre wide, 30 m long belt transect, oriented North to South ran from a stand of tall (≥1.5 m) multi-stemmed semi-prostrate *K. ericoides* var. *microflora* to an area of scattered low lying *K. ericoides* var. *microflora* shrubs adjacent a thermal lake. Further one metre wide, 100 m long transects were investigated, these ran parallel to each other ten metres apart. The transects orientated from East to West down the slope of the foothills of the Paeroa scarp from an area of highly active steaming ground onto flat ground at the base with little geothermal activity.

Vegetation was recorded and its height was measured every two metres along the 30 m transect and every five metres along the 100 m transects, as well as whenever a sporocarp was present. Soil temperatures and 25 mm diameter soil cores were taken from the beneath the vegetation canopy and the base of the sporocarp (if present). The cores were wrapped in plastic bags to try and keep them intact and they were brought back to the laboratory and stored at 4°C until analysis of the root zone and ectomycorrhiza was carried out.

Intact soil cores were used to measure the maximum root depth of fresh roots in the organic mat and mineral soil, from that the relative abundance of roots in each zone was calculated. The soil cores were saturated with an equal volume of distilled water and left to equilibrate for 24 hours before the pH was analysed. The level of *P. tinctorius* association was determined by the percentage of infected roots to uninfected roots in five replicate sub-samples from each core.

## RESULTS

Soil temperatures in thermally active areas at Te Kopia rise rapidly with depth; a typical soil temperature profile has a gradient from 28°C at the surface to 76°C at 150 mm ($r^2$ = 0.97; P = 0.001). This profile is vertically stratified into two discrete zones, the organic mat and the mineral soil. Beneath a canopy of prostrate kanuka organic material and moss up to 50 mm deep serves as a root habitat and condensation point for water vapour moving out of the heated soil. Beneath this layer the white/grey mineral soil is largely devoid of organic matter. Where roots penetrate the mineral soil they do so to a depth of 13.1 mm. The majority of roots grow in the organic mat, although, in cooler areas the roots enter further into the mineral soil but go no deeper than 50 mm below the base of the organic mat. The maximum root depth is negatively related to the 50 mm soil temperature, which shows a range from 15 mm at temperatures of 55°C to 59 mm deep at 36°C ($r^2$ = 0.80; P< 0.05). The depth of organic mat on top of the soil has a strong positive relationship with the height of plants above (r2 = 0.49; p < 0.02).

The height of prostrate kanuka varied widely at Te Kopia with plants ranging from sprawling plants 100 mm tall up to 2.6 m tall semi-prostrate trees; however, most plants were no taller than 1.2 m. Belt transects were used to sample a discontinuous canopy of prostrate kanuka, with an average height of 455 mm (s.d. = 328) and average cover of 51.0 % (s.d. = 32.7). As is suggested by the relatively large standard deviations there is a wide variation in both plant height and canopy cover which are positively correlated (p< 0.01). The height prostrate kanuka had no relationship to the pH of the Te Kopia soils.

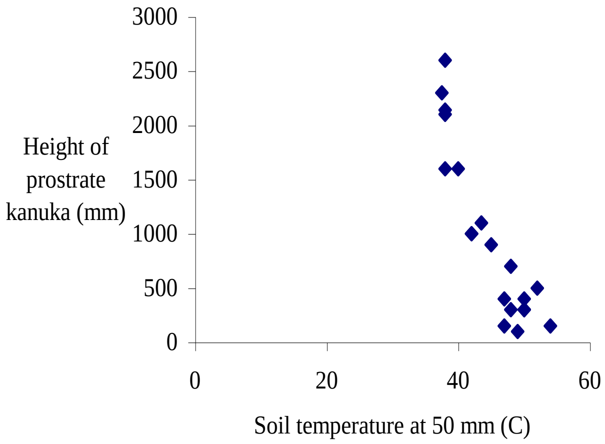



**Figure 1** The relationship of the height of *K. ericoides* var. *microflora* with soil temperature at 50 mm depth along TN1 (R2 = 0.91; P<0.001; n = 18).

TN1, the 30 metre transect that traversed the base of the steamfield, was in an area of low thermal activity following a gradient in plant height. The ground in this area was consistently level and the soil was sandy silt. The heights of prostrate kanuka measured along the 30 m transect ranged from 30 mm to 2.6 m tall; the wide range in plant heights found here illustrates well the relationships between the growth form of prostrate kanuka and soil temperature (Figure 1). Semi-prostrate *K. ericoides* grows on cooler soils and as soil temperature increases along gradient the height of the prostrate kanuka rapidly decreases to below 500 mm at 50°C ($R^2$ = 0.918; P<0.001; Figure 1).

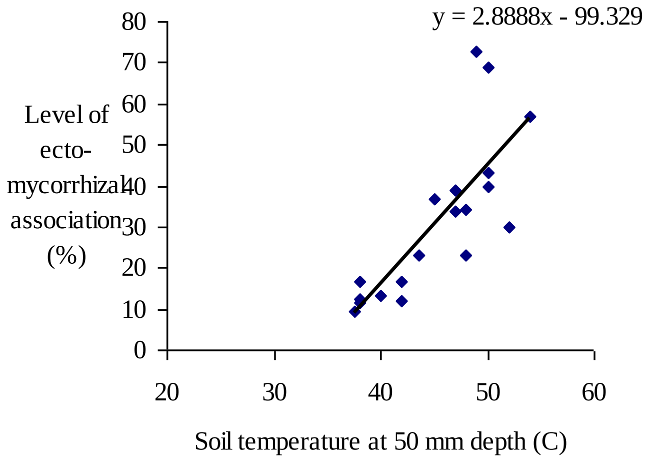



**Figure 2** The relationship between the level of ectomycorrhizal association between *P. tinctorius* and soil temperature along TN1 ($p<0.001$).

Soil temperature has a strong influence on both the height of prostrate kanuka and the abundance of *P. tinctorius* ectomycorrhiza along the TN1 transect. The rapid decline in the height of plants along TN1 in relation to increasing temperatures at 50 mm (Figure 1) is counter to the trend observed between temperature and the abundance of *P. tinctorius* ectomycorrhiza which demonstrates a strong positive relationship (Figure 2). A strong negative relationship ($p<0.001$) between the abundance of *P. tinctorius* and the height of prostrate kanuka (Figure 3) shows that the taller plants have a lower level of ectomycorrhiza than those shorter plants which have up to 70% of their feeder roots forming ectotrophic associations.

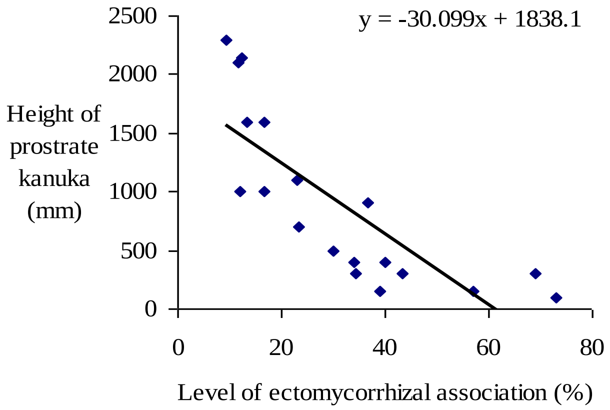
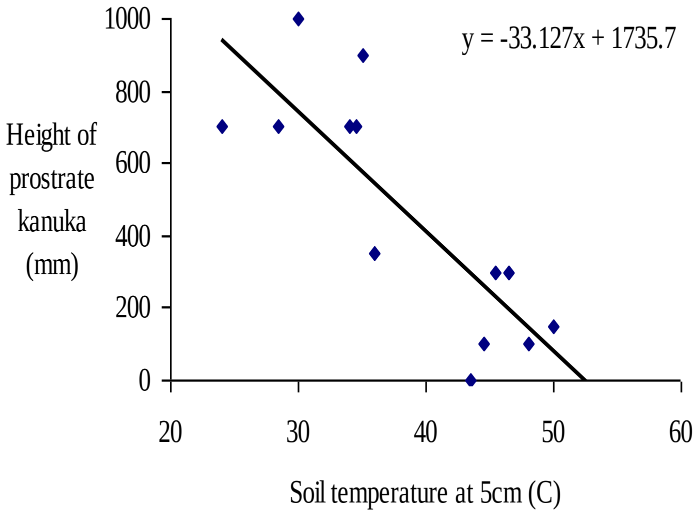



**Figure 3** The relationship between the level of *P. tinctorius* ectomycorrhiza and the height of *K. ericoides* var. *microflora*

The other transects sampled were carried out ten metres apart, running down the scarp, perpendicular above TN1. The substrate of these transects was variable, and unstable in places on the slope and finished on similar ground to that of TN1. Plants measured along these transects do not have the height range that was observed along TN1, as no plants measured exceeded 1 m in height, which is typical of most stands of prostrate kanuka. When grouped these transects showed no trend but when analysed individually trends were evident. For the purpose of this report TN2 will be used as an example.

a



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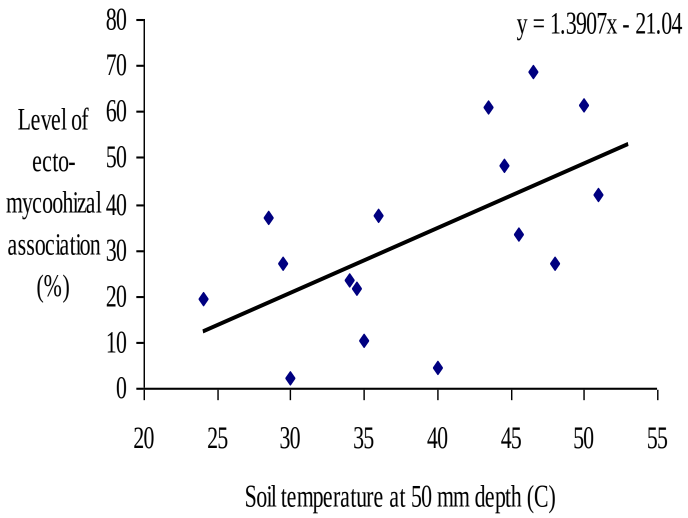


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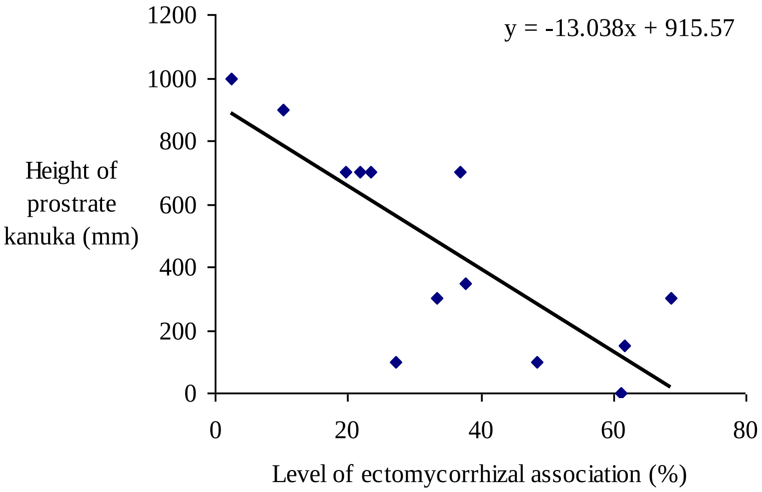
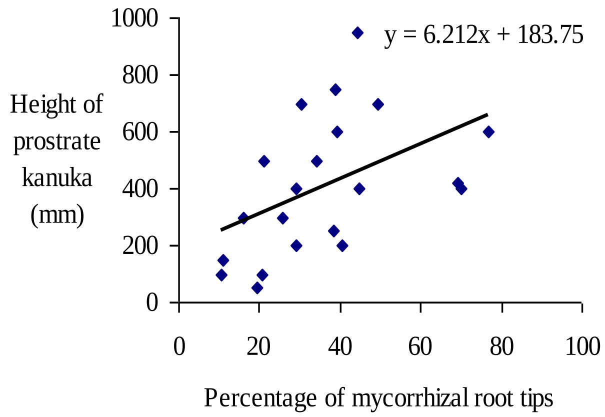



**Figure 4** TN2 relationships; a) *K. ericoides* var. *microflora* height and 50 mm soil temperature, b) level of mycorrhizal association and soil temperature at 50 mm and c) height of *K. ericoides* var. *microflora* and level of mycorrhizal association.

The trends for TN2 support those found in TN1, although the correlations are slightly weaker. The relationship between the height of prostrate kanuka and soil temperature at 50 mm depth, along had a strong negative trend ($p<0.001$; Figure 4a). A positive relationship between soil temperature and the level of *P. tinctorius* ectomycorrhiza was




found (p = 0.001, Figure 4b), as was a negative relationship between plant height and the percent of mycorrhizal association (Figure 4c).

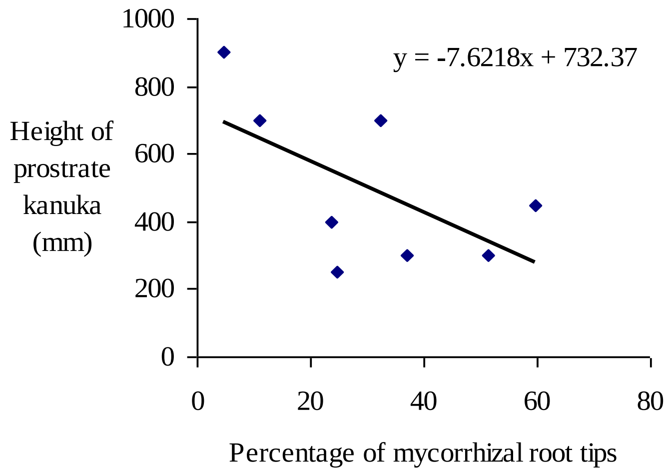



**Figure 4.15** Height of *K. ericoides* var. *microflora* and level of mycorrhizal association found on the slope (a) and on the flat (b).

Of the 100 metre transects TN4 had no apparent link between temperature and height of prostrate kanuka ($p>0.05$) or between temperature and the level of ectomycorrhizal association ($p>0.05$). Furthermore, there was no relationship between the level of association and the height of prostrate kanuka. However, the transect ran through two very different environments. If the transect is divided into these two distinct zones, the slope and the flat, two different trends appear (Figure 5). Prostrate kanuka was typically shorter on the slope than plants of the same species growing on the flat ground ($p<0.05$) and there is a strong positive relationship between prostrate kanuka height and the abundance of *P. tinctorius* ectomycorrhiza ($p<0.05$; Figure 5a). The relationship between the level of *P. tinctorius* ectomycorrhizal association and the height of prostrate kanuka growing on the flat ground is negative ($p=0.116$; Figure 5b). The growth habit on this slope contradicts those previously found and illustrates gains in the growth of prostrate kanuka from increased *P. tinctorius* infection and suggests that temperature is not the most influencing factor in this area, as it is in others.

## DISCUSSION

The confirmation of the ectomycorrhizal association between *Pisolithus tinctorius* and prostrate kanuka is the first step in the understanding of relationships between the vegetation and the soil micro organisms of geothermal environments. Although *P. tinctorius* has been documented in New Zealand and *Kunzea* has been suggested as the host, there has previously been no published confirmation of potential native host plants (Cunningham, 1944; Chu-Chou & Grace, 1983; Marx, 1977; Cairney & Chambers, 1997). This study provides the first confirmation and synthesis of a *P. tinctorius* ectomycorrhizal association with native tree species in New Zealand, in particular with prostrate kanuka, which naturally forms an association in extreme geothermal environments.

The benefits of prostrate kanuka forming an ectomycorrhizal association with *P. tinctorius* are essential to its survival on geothermal soils. Mycorrhizal associations increase the nutrient absorption by plants by increasing the surface area to volume ratio of the uptake system (Li *et al.* 1991). The enhanced efficiency of resource acquisition by mycorrhizal plants allows more energy to be allocated to growth and reproduction that increases plant fitness (Pedersen and Sylvia, 1996). This is very important on acid soils where minerals, particularly phosphorus, are occluded. In addition, in many ecosystems, plants and mycorrhizal fungi are exposed to a wide variety of toxic compounds and it has been suggested that mycorrhizal fungi may effectively mediate and alter the interaction between plants and these compounds (Berry, 1982; Pedersen and Sylvia, 1996). The mediation of toxic compounds present in lethal concentrations in the geothermal environment is essential (Burns, 1997).

*Pisolithus tinctorius* has been recorded in habitats throughout the world, primarily as an early coloniser of mineral soils and is commonly present in heavily eroded and mine-site soils, which have low pH, high temperatures (above 19°C) and toxic concentrations of minerals (Brundrett, 1991; Cairney and Chambers, 1997; Gardner and Malajczuk, 1988; Marx, 1977). The geothermal environment was found to have soil conditions at the extreme of other habitats previously described, with pH as low as 2.7 and soil temperatures up to 55°C in stands of prostrate kanuka at Te Kopia.

Temperature is a central component in the relationship between the abundance of *P. tinctorius* ectomycorrhiza and any benefit derived from the association by the host plant. Plants inoculated with *P. tinctorius* growing in soils with temperature below 19ºC either failed to form associations or exhibited infectivity as low as 4% (Brougher *et al*, 1990; Pons *et al*. 1986). However, the level of mycorrhiza developed between *P. tinctorius* and *Pinus mugo* grown *in vitro* rose form 3% at 23ºC to 35% mycorrhizal root tips at 33ºC (Pons *et al.* 1986). The level of *P. tinctorius* association with prostrate kanuka at Te Kopia supported these findings, forming a strong positive correlation with the soil temperature.

Associations with *P. tinctorius* have been shown to provide exceptional improvement in growth for the host plant. *Pinus* and *Eucalyptus* species in mine rehabilitation and reforestation trials inoculated with isolates of *P. tinctorius* had up to 585 % increase in height (Marx, 1977; Berry, 1982; Cairney and Chambers, 1997). Contrary to this, infection with *P. tinctorius* did not confer any benefit to prostrate kanuka with regards to height. The heights of the plants were strongly correlated to soil temperature, which meant hotter soils placed a greater stress on the plants, restricting their growth and stimulating an increase in the percentage of *P. tinctorius* ectomycorrhizal roots. This suggests that the association is necessary for prostrate kanuka to survive in the more extreme areas and therefore illustrating the obligatory nature of this symbiosis.

In geothermal areas, typified by the Te Kopia field, the soil conditions have been shown to be beyond that which can support the survival of the usual mycorrhizal symbionts of *K. ericoides*. A lack of association with other fungi found in the field surveys and the inability of the *Glomus* inoculum to form a mycorrhizal association in the geothermal soil suggests that there is a competitive advantage for *P. tinctorius* in the high stress soils (Bridge, 2001). In mixed inoculum trials grown in forest soils, *P. tinctorius* was not competitive, which was attributed to its slower experimental growth rate (McAfee and Fortin, 1988). Organisms tolerant of high stress environments have superior growth in these conditions but are readily out competed under 'normal' soil conditions (Brundrett, 1991; McAfee and Fortin, 1986). This is supported by the lack of reported *P. tinctorius* outside geothermal areas in New Zealand and its inability to out compete local soil fungus in non-geothermal soil trials (Bridge, 2001). Because the mycorrhizal association is obligate for the fungus (Hacskaylo, 1971) and it has been shown that under high stress prostrate kanuka has an increased mycorrhizal infectivity, the association between *P. tinctorius* and prostrate kanuka appears to an obligatory one.

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