Pinus patula establishment problem associated with poor ectomycorrhizal development in previously cultivated soils

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SYNOPSIS

A field survey of Pinus patula *Schlecht. et Cham. plantations at Mondi's Mooi River Estate, Kwazulu-Natal, South Africa was conducted to assess the levels of ectomycorrhizal (EM) fungal colonisation. The objective was to determine whether the establishment problem (old land syndrome) is related to the quality and quantity of EM fungal colonisation of* P. patula *roots. EM colonisation was assessed in previously cultivated soils and compared to that in virgin soils at four sites. Mycorrhizal colonisation was higher in roots from virgin soil than in previously cultivated soils, although this difference was statistically significant at only two sites. Basidiomata of three EM fungi* (Thelephora terrestris, Laccaria laccata *and* Boletus edulis) *were collected from the plantations in virgin soil and one* (T. terrestris) *from the previously cultivated soil. Three types of EM fungal roots were identified from the survey area. These findings suggest that poor mycorrhizal development in previously cultivated soils could be an important factor contributing to establishment problems in these soils.*

KEY WORDS: Pinus patula, old land syndrome, ectomycorrhiza

INTRODUCTION

During the last decade, the timber industry has expanded considerably in South Africa. Land utilised for forestry extends over an area of 1,5 million hectares (Van der Zel, 1994). Large areas of virgin grasslands and lands that were previously cultivated with agronomic crops are today planted with exotic trees such as *Pinus* spp., *Acacia* spp. and *Eucalyptus* spp.

Establishment of *P. patula* on lands that were previously cultivated to maize, wheat and sugar cane has been poor, whereas its establishment on native grasslands has mostly been successful (Schumann and Nobel, 1993). This problem of poor establishment has been commonly referred to as "old land syndrome", it is characterised by stunted shoot and root growth, chlorosis and stunting of fascicles, loss of apical dominance and early mortality of transplanted seedlings. A similar pine establishment problem on previously cultivated soils (PCS) has been reported from the U.S.A. (Steinbeck, 1990; Mitchell *et al.*, 1991).

From 1990 to 1994, a series of investigations were carried out to study the role of fungal pathogens, nematodes, soil physical factors, nutrient status, herbicide residues and allelochemicals in the pine establishment problem (Schumann *et al.*, 1994). These studies reported improved pine growth with nitrogen fertilisation, soil sterilisation and scalping of the top soil layer, indicating nutritional as well as biological factors in the old lands. Isolation of several genera of *Pythium* and *Phytophthora* species from PCS and pine roots in these soils has led to the conclusion that root disease may be another major factor causing pine mortality (Linde *et al.*, 1994). Similarly, there has been speculation that an overgrowth of weeds in the PCS may suppress pine growth either through direct competition for nutrients, or through the suppressive allelopathic effect of chemicals that such weeds can produce (Reinhardt *et al.*, 1996).

The positive effect of EM fungi on plantation regeneration has been well documented (Amaranthus, 1992). It is often considered difficult, and sometimes even impossible to plant pine seedlings into new or disturbed areas without the introduction of the symbiotic mycobiont (Bledsoe, 1992). EM fungi improve the health and development of their hosts by enhancing plant nutrition, improving soil structure and disease resistance and increasing stress resistance (Paul and Clark, 1989). Since poor soil structure, nutrient deficiency and soil- borne pathogens are prevalent in these PCS (Schumann *et al.*, 1994) a beneficial effect of mycorrhization can be expected.

Although mycorrhizal suppression in PCS has been considered by previous researchers as another possible factor involved in the old land syndrome of pine (Schumann *et al.*, 1994), a quantitative assessment of EM fungi has not previosly been conducted. This study investigated the extent and type of

P. patula EM fungal colonisation in virgin soil and PCS, with the objective of determining the importance of EM as a factor in the old land syndrome.

MATERIALS AND METHODS

Two surveys were conducted in *P. patula* forests at Mondi Estate, Mooi River, Kwazulu-Natal, South Africa. Four sites (designated as AO2, AO3, BO7 and C17/18) were selected for sampling, where virgin soil (VS) and PCS were situated adjacent to each other and each site was planted with *P. patula* trees of the same age (site C17/18 planted in Jan. 1989 and the rest of the sites in Jan. 1991). The predominant soil type at these sites was Hutton.

Survey I

The first survey was conducted during May, 1996. In a completely randomised design, five trees were selected for sampling from VS and five from PCS, at each site. Each sample was taken by excavating 60 cm^3 of soil close to the base of the tree with a shovel. Mycorrhizal roots were collected from the top litter layer and below ground to a depth of 60 cm along with the adhering soil. Each sample was placed separately in a plastic bag and kept in a cooler. Subsequently the whole sample was immersed in water for 1h to loosen the roots from the soil. Soil aggregates were gently crumbled by hand, and root fragments were recovered by sieving through a 140µm (100 mesh) sieve. After rinsing under running tap water, cleaned root fragments captured on the sieve were transferred to containers with FAA (90 ml 50% ethanol + 5 ml formaldehyde + 5 ml acetic acid) and transported to the laboratory.

To quantify EM colonisation the whole root mass from each sample was cut into 1 cm long pieces. Fifty randomly selected lateral root pieces were placed in a Petri dish with water and the number of monopodial, bipodial and coralloid roots per cm was counted under a stereo-microscope, at 10 x magnification. Subsequently the mycorrhizal tips were detached from the same lateral roots and the total number of mycorrhizal tips per cm of root was counted.

A linear model was used with fixed effects for location, soil and interaction between location and soil. A random effect for the plants was included for each plant within a specific location and soil. To test for significance of the effects included in the model, the SAS (Statistical Analysis System, SAS Institute Inc., 1982) procedure GLM was used. To test for the significance of the difference between means the option in the GLM procedure to specify specific contrast was used.

Survey II

Basidiomata of EM fungi are normally produced from late October to early February in the Kwazulu-Natal area. Hence a second survey of the same plantation was conducted in November 1996 to sample EM roots and associated basidiomata.

Basidiomata were collected from the above mentioned sites and soils, except PCS at sites AO3 and BO7 where, by the time of the second survey, dying *P. patula* trees had been removed and soils planted with eucalypts.

A thorough search for fungal sporocarps was made, and samples were collected for examination. Fresh sporocarps, within three hours of collection were used for making spore prints while additional sporocarps were preserved by air drying. These sporocarps were identified on the basis of keys of Reid and Eicker (1991) and Van der Westhuizen and Eicker (1994).

Pine EM under each basidioma were dug within approximately 60 cm radius of the basidioma to determine the structure and morphology of the mycorrhizae formed. Fresh and Trypan bluestained roots were examined for morphological characteristics such as colour, EM type, rhizomorphs and attached mycelium under a stereo-microscope. For anatomical studies, mycorrhizal root tips were fixed in FAA for 24h and dehydrated by the butanol alcohol method (Sass, 1964). The fixed samples were impregnated and embedded in paraffin wax. Thin sections of 5-6 µm thickness were cut by means of a rotary microtome, stained differentially with safranine and fast green, mounted in Canada Balsam and examined under a Leitz Ortholux microscope to determine thickness of the mantle and Hartig net penetration into the cortex.

Fifty roots from the vicinity of each basidioma were cleared of soil and debris and examined under a stereo-microscope to determine the percentage distribution of EM root type associated with a particular basidioma. However, where basidiomata were not found within the sampling area, roots were taken at random from the VS and PCS to determine percent distribution of EM types in those soils. Root tips were also squashed on a microscope slide, stained with trypan blue and examined under a Leitz Ortholux microscope for EM fungal mycelium with clamp connections, emanating hyphae and mycelium of other fungi growing on the root surface.

Nursery seedlings EM colonisation

To evaluate the initial EM colonisation status of *Pinus patula* seedlings, before they are planted in VS or PCS, twenty five seedlings (five months old) were selected at random from five seedling trays at Mondi Nursery (5 seedlings/tray- containing a total of 98 seedlings). Roots of the seedlings were processed, quantified and characterised for EM fungal colonisation as described in the methods for survey I.

RESULTS

An overall analysis of variance of the four sites (total across all sites) indicated that the total number of EM

tips per cm of roots was significantly different ($p<0,001$) among sites and that total EM colonisation was higher in VS ($p<0,01$). At every individual site, total EM colonisation was higher in all VS, but the difference was significant only at site BO7 and AO2 (Table 1).

At site AO2 and BO7 the mean number of branched coralloid roots (>5 tips) was significantly higher ($p<0,01$, $p<0,001$) in VS, whereas the number of unbranched monopodial roots was significantly higher at site BO7 in PCS ($p<0,001$).

Three different types of ectomycorrhizal roots were recorded in the detailed analysis of ectomycorrhizae. These EM types varied in morphological characteristics such as short roots, branching pattern, colour and dimensions. Anatomical studies showed differences among the three types in thickness of the mantle and penetration of the Hartig net into the cortex (Table 2 and Fig. 1-3).

TABLE 1. Extent of EM colonization of P. patula *roots in VS and PCS*

Site ‡		Ectomycorrhizal tips/cm root				
		Mono	Bi	C 3-5	C >5	Total
AO2						
	PCS	0,08† (0,03)	4,08 (0,28)	2,20 (0,18)	0,23 (0,07)	11,10 (0,42)
	VS	0,04 (0,02)	2,20 (0,14)	1,35 (0,14)	2,16 (0,19)	18,82 (0,94)
	Contrast	NS	NS	NS	*	*
AO3						
	PCS	0,03 (0,10)	1,15 (0,13)	2,67 (0,17)	0,89 (0,11)	13,25 (0,54)
	VS	0,00	1,75 (0,26)	2,48 (0,19)	1,78 (0,15)	18,76 (0,66)
	Contrast	NS	NS	NS	NS	NS
BO7						
	PCS	1,45 (0,13)	1,46 (0,18)	1,21 (0,19)	0,51 (0,13)	8,02 (0,80)
	VS	0,38 (0,00)	1,44 (0,17)	1,09 (0,16)	2,48 (0,20)	19,05 (1,04)
	Contrast	**	NS	NS	**	**
C17/18						
	PCS	0,13 (0,05)	2,72 (0,20)	1,08 (0,12)	0,99 (0,14)	11,42 (0,72)
	VS	0,08 (0,04)	1,20 (0,16)	1,77 (0,10)	1,63 (0,15)	15,52 (0,65)
	Contrast	NS	NS	NS	NS	NS
Ectomycorrhizal tips/cm root						
F-Statistics	**df**	Mono	Bi	C 3-5	C >5	Total
Site	3	NS	**	NS	NS	***
Soil	1	**	NS	NS	***	**
Site * Soil	3	*	NS	NS	NS	NS

***, **, * Significant at p = 0,001 0,01 0.05

NS = Not significant.

† Mean of 250 root pieces (SE of the mean).

‡ Designation of site at Mondi Estate, Mooi river, Kwazulu-Natal.

PCS = Previously cultivated soil, VS = Virgin soil.

Mono = monopodial, Bi = bipodial, C = coralloid.

TABLE 2. Morphological and anatomical characteristics of three EM types collected from VS and PCS

Characteristic	EM Type		
	I	II	III
Morphology	Mono & bipodial	Mono, bi & coralloid	Mono & bipodial
Colour	White	Light yellow to pale brown	Yellow to dark
Surface	Rough	Cottony	Smooth
Length x diam(mm)	1,5-2 x 0,5-0,7	1,5-1,6 x 0,4-0,6	3,5-4 x 0,4-0,5
Rhizomorph	Few, light brown	Abundant, buff brown	Very few, brown
Mantle (µm)	7-10	28-32	12-15
Hartig net penetration into cortex	1 layer	3 layers	1-2 layers

TABLE 3. Percent distribution of EM root types in the soil adjacent to the basidiomata

Site	EM species (basidioma)	EM type (%)		
		I	II	III
AO2				
PCS	*T. terrestris*	0	20	80
VS	*L. laccata*	35	40	25
	T. terrestris	10	90	0
AO3				
PCS	ND	ND	ND	ND
VS	*L. laccata*	45	55	10
	T. terrestris	24	76	0
BO7				
PCS	ND	ND	ND	ND
VS	*B. edulis*	25	15	60
	T. terrestris	25	52	23
C17/18				
PCS	None	35	0	65
VS	None	90	10	0

Designation of site at Mondi Estate, Mooi River , Kwazulu-Natal.
PCS = Previously cultivated soil, VS = Virgin soil, ND = Not determined.
I, II, III = Three types of EM as explained in the results.

Distribution of EM basidiomata varied within sites (Table 3). At site AO2 (VS), several small basidiomata of *L. laccata* were found underneath the leaf litter, and two basidiomata of *T. terrestris* were found. A mixture of EM root types was associated with *L. laccata* i.e. 40% type II, 35% type I and 25% type III, whereas 90% of the roots associated with the two *T. terrestris* basidiomata were of type II Few *T. terrestris* basidiomata were recorded at the same site in previously cultivated soil, but the majority of roots (80%) were of type III. Several basidiomata of *L. laccata* and *T. terrestris* were present in the VS at site

AO3. The majority of roots associated with these two types of basidioma were of type II (55-76%) and type I (24-45%), with only 10% of type III. *Boletus edulis* and *T. terrestris* occurred at site BO7. Of the roots associated with *B. edulis* 60% were of type III, 25% of type I and 15% of type II. Of the roots associated with *T. terrestris* 52% were of type II and 23-25% of the other two types. At site C17/18 basidioma were absent, but the virgin soil contained mostly type I mycorrhizal roots whereas previously cultivated soil contained 65% type III roots and 35% type I roots.

Ectomycorrhizae were not only abundant but also the main colonisers of roots in VS (Table 4). Fifty to 100% of the roots in VS had mycelium with clamp connections and EM fungal hyphae emanating from the mantle. Roots in the PCS had a diverse population of fungal mycelia mostly from non-mycorrhizal origin, and only 20-30% roots had EM type mycelium.

Nursery seedlings were 100% EM-colonised with a mean of 4,22 EM tips per cm of root (data not shown). EM roots were mostly monopodial or bipodial and were similar to type II EM roots in morphological and anatomical characteristics.

TABLE 4. Percentage of roots with EM and other mycelial growth in the VS and PCS

Site	EM mycelium	Clamp connection	Emanating hyphae	Other fungal mycelium
AO2				
PCS	20	0	0	60
VS	100	50	60	0
AO3				
PCS	ND	ND	ND	ND
VS	60	40	50	50
BO7				
PCS	ND	ND	ND	ND
VS	80	40	40	0
C17/18				
PCS	30	20	0	60
VS	60	20	0	60

Designation of site at Mondi's Mooi River Estate, Kwazulu-Natal.
PCS = Previously cultivated soil, VS = Virgin soil, ND = Not determined.

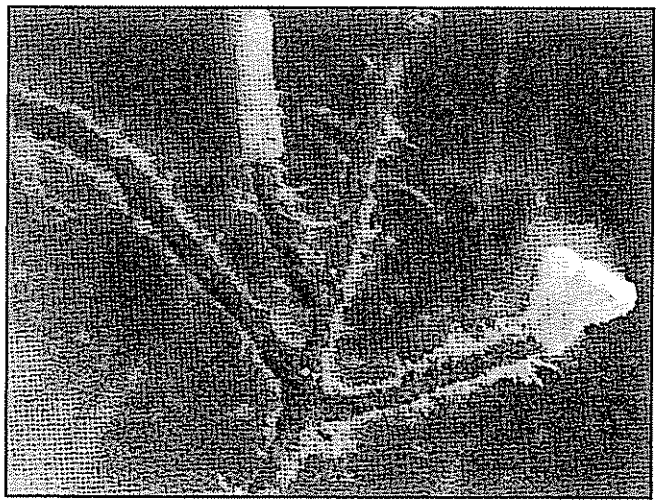

FIG. 1a: Root morphology of type I mycorrhiza (10 x)

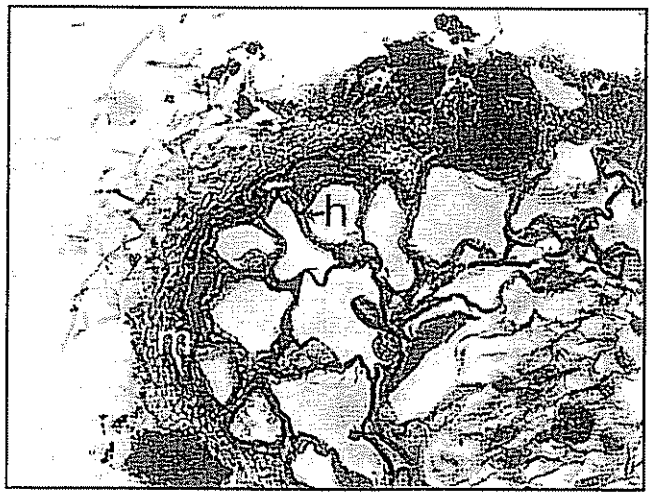

FIG. 1b: Longitudinal section of type I mycorrhiza (400 x) showing the mantle (m) and Hartig net (h)

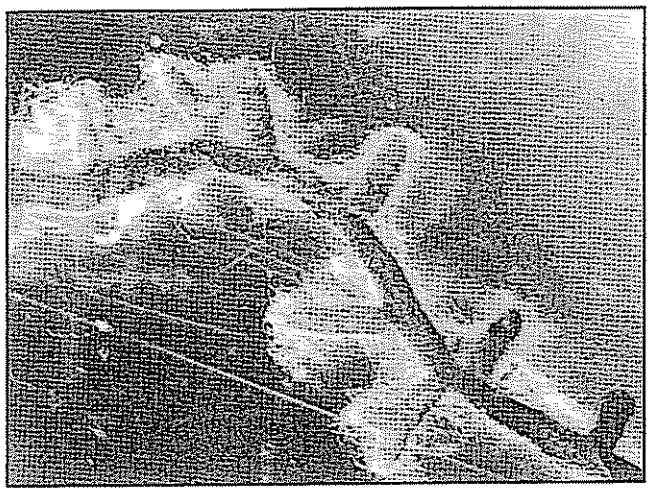

FIG. 2a: Root morphology of type II mycorrhiza (10 x)

FIG. 2b: Longitudinal section of type II mycorrhiza (400 x) showing the mantle (m) and Hartig net (h).

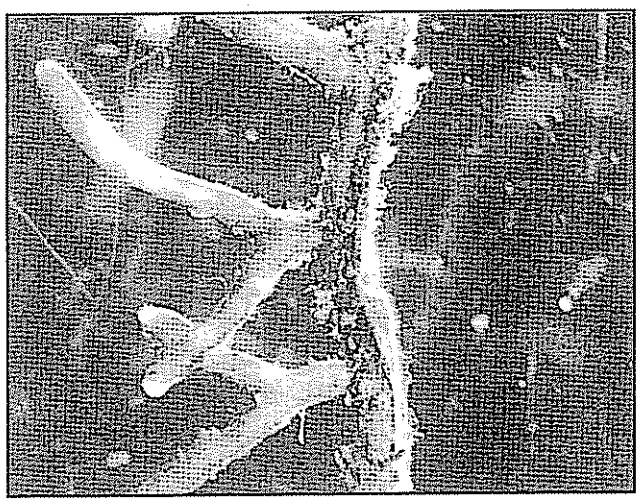

FIG. 3a: Root morphology of type III mycorrhiza (10 x)

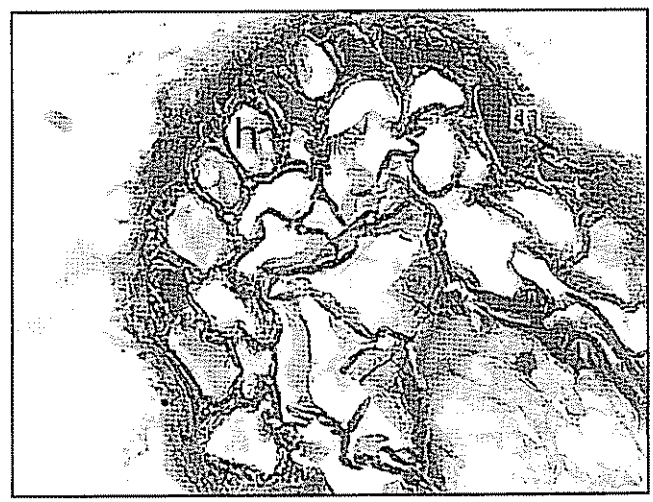

FIG. 3b: Longitudinal section of type III mycorrhiza (400 x) showing the mantle (m) and Hartig net (h).

DISCUSSION

It is evident from the results that previously cultivated lands were deficient in EM fungal colonisation. A higher number of monopodial, and insignificantly different bipodial roots in PCS indicates that roots were mostly colonised by *T. terrestris*. However, a significantly higher number of total EM tips and coralloid roots in VS indicate that EM roots in VSs were not only extensively colonized by EM fungi but this branching pattern of short roots also indicates a diverse group of EM fungi present in VS.

Nineteen species of EM fungi are reported from pine-growing areas in South Africa (Van der Westhuizen and Eicker, 1987). Of these species basidiomata of only three were collected from the VS and one from PCS in the present study. Three distinct types of EM roots were observed on the basis of morphological and anatomical characteristics. Type I roots were unique in their characteristics and to the authors' knowledge do not match with the description of any previously described EM root types associated with *P. patula*. Type II characteristics correspond with the description for *T. terrestris* (Van Greuning and Van der Westhuizen 1990; Mohan *et al.*, 1993) and type III with that of *B. edulis* (Marais and Kotzé, 1977).

Since this was a one- time sampling it is conceivable that other basidiomata which are produced during other times of the year, under different environmental conditions, may occur. Also, due to a limited number of basidiomata assessed, a clear correlation between mycobiont and EM root type could not be determined.

The finding that *P. patula* seedlings from the Mondi nursery (grown in pine bark medium) were abundantly colonised by *T. terrestris*, corresponds with a previous report based on a survey conducted in the eastern Transvaal (Van Greuning and Van der Westhuizen, 1990). Our data, therefore, indicate that after these *T.terrestris*-colonised seedlings were transplanted into PCS, the subsequent EM colonisation and the pattern of succession differed from that in VS. There are numerous factors which could conceivably cause such a different pattern in PCS, namely absence of EM inoculum, a soil environment hostile

to EM development, differences in soil micro-flora and the presence of pathogens which prevent root development.

Our findings indicate that *T. terrestris*, being an aggressive coloniser, survived in PCS, whereas other EM fungi partially or completely replaced it in VSs. *T. terrestris* and *L. laccata* are reported to be less dependent on their hosts for nutrition (Griffiths and Caldwell, 1992). In contrast with other types of EM fungi which are often entirely dependent on their hosts for carbon supply, these fungi often die off or form resting spores as the energy supply from the host is reduced or interrupted. It is, therefore, possible that *P. patula* seedlings are weakened in PCS, consequently favouring *T. terrestris* and *L. laccata* colonization.

PCS at Mondi's Mooi River Estate are densely populated with weeds, predominantly *Conyza sumatrensis, Bidens pilosa* and *Tagetes minuta*, which are reported to have detrimental effects on mycorrhizal development in *P. patula* seedlings (Reinhardt *et al.*, 1996). An inhibitory effect of weeds on EM of black spruce (Mallik and Zhu, 1995) and conifers (Robinson, 1971) has also been reported. Hence, weeds could be one of the factors contributing to the poor EM development in PCS.

The reported involvement of soil-borne pathogens such as *Pythium* spp. in the old land syndrome (Linde *et al.*, 1994), together with our data showing comparatively poor EM establishment in *P. patula* roots in PCS compared to that in VS, and the reported inhibitory effect of weeds on EM development, suggests a tripartite EM-weed-pathogen interaction involved in the old land syndrome. It is possible that *P. patula* seedlings planted into PCS are weakened because of the absence of efficient EM fungal symbiont to promote growth and protect the roots against pathogens, thereby making pine seedlings particularly vulnerable to attack by soil-borne pathogens such as *Pythium* spp.

South African foresters have given little attention to mycorrhizal efficiency, probably because *T. terrestris* occurs naturally throughout local pine plantations (Van der Westhuizen and Eicker, 1987). It has not been generally realised that the mere presence of mycorrhizal fungi is of lesser importance than the efficiency and compatibility of the mycorrhizal symbiont. To our knowledge none of the EM fungi found in the area have been evaluated and reported in literature to be efficient symbionts. Indeed, *T. terrestris* has been reported to be an inefficient symbiont (Marx and Bryan, 1970). Therefore, in future research the introduction and establishment of efficient EM fungi into PCS should be considered as an essential component of managing the old land syndrome of pines.

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