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Role of Mycorrhizae, Pathogens and Weeds in Sustainable Pine Forest Management

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ABSTRACT

Greenhouse studies were conducted to determine the allelopathic effect of weeds (*Bidens pilosa, Conyza sumatrensis, Tagetes minuta)* on infection of pine roots by pathogenic *Pythium spp.* and symbiotic ecto-mycorrhizal (EM) fungi and subsequent mortality and growth suppression of pine seedlings after transplantation into previously cultivated and virgin soils. Results showed zero incidence of *Pythium* in pine roots grown without weeds; whereas, treatments with weeds showed more than 40% incidence of this pathogen in pine roots from both previously cultivated and virgin soils. EM colonization was significantly suppressed in the presence of weeds. The data suggests that seedlings were predisposed by a stress factor in the presence of weeds rendering them more susceptible to pathogen infection. It is probable that poor EM colonization in the presence of weeds rendered the seedlings more vulnerable to infection by soil born pathogens. Further research would be required to confirm these probabilities, but a better management of weeds and pathogens and pre-inoculation of seedlings with effective EM types may be a solution towards sustainable pine afforestation in previously cultivated soils.

Key Words: Old land syndrome; *Pinus patula; Pythium;* Ectomycorrhizae

INTRODUCTION

Pinus patula has been planted extensively for pulp, wood and fibre production in South Africa. To increase the area of production, it is a common practice to establish forests on lands that were previously used for cultivation of agricultural crops (so called old lands). Since inception, *P. patula* plantations showed poor growth and survival on these lands, while plantations of the same tree species grew successfully on the nearby virgin grasslands (Schumann & Noble, 1991). Although economic losses due to this problem have not been estimated, its effects are quite devastating. In 1989, 20,000 hectares of old lands were planted with *P. patula* by Mondi Pty. Ltd. in the Natal region of South Africa and losses up to 95% and higher were experienced (Linde *et al*., 1994). Similar problems were experienced in the North-Eastern Cape, where even a second and third replanting resulted in total failure (Linde *et al*., 1994). This phenomenon has been observed by several researchers within South Africa (Schumann & Noble, 1991; Atkinson & De Beer, 1992; Smith & Van Huyssteen, 1992; Linde *et al.,* 1994) and in the U.S.A. when afforestation of old-fields with *P. patula* was attempted (Steinbeck, 1990; Mitchell *et al*., 1991). The problem has been identified as "old field syndrome", having the symptoms of chlorosis and necrosis of the terminal ends of seedling needles, dwarfed seedlings, loss of apical dominance and poor root development which are apparent four to five months after seedlings transplantation into the old lands.

Pathogenic fungi such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Trichoderma spp*, *Pythium* spp and *Phytophthora* spp. have been isolated from roots of *P. patula* grown in old land soils (Lundquist, 1987a,b). *Pythium* spp were considered as the major causal factor in the old land syndrome by Linde *et al*. (1994). Recently, it has been observed that old lands are thickly populated with weeds such as *Conyza sumatrensis*, *Bidens pilosa* and *Tagetes minuta* (Reinhardt *et al*., 1996). It was hypothesized that competition for light and nutrition and weed allelopathic effect on pine and symbiotic organisms associated with pine (ectomycorrhizae, EM) might be the additional factors involved in the old land syndrome. EM fungi form a symbiotic association with *P. patula* and are reported to protect the plant against pathogens, improve nutrient uptake, stimulate growth (Schenck, 1981) and detoxify allelochemicals from weeds (Mallik & Zhu, 1995). The role of EM in the old land syndrome has not been previously investigated.

The present study was undertaken to investigate the effect of weeds on the infection and colonization of pine roots with pathogenic (*Pythium*) and symbiotic (EM) fungi, and subsequently the effect of these interactions on pine growth in old land soils.

MATERIALS AND METHODS

P. patula seedlings (3-month-old) were obtained from Mondi nursery, Pietermeritzburg, Kwazulu-Natal. Prior to planting, roots of these seedlings were tested for the presence of *Pythium* spp. by plating onto a selective medium (Mitchel & Kannwischer-Mitchell, 1992). The seedlings were also assessed for naturally occurring EM by directly counting the number of EM root tips cm^{-1} of root. Soil was collected from Mondi estate at Mooi river,

Kwazulu-Natal, South Africa. Three sites (block numbers CO9, AO3 and C17/18) were selected where both old land and virgin soils occurred adjacent to each other in each block. Ten topsoil sub-samples (approximately 3 kg) were excavated upto about 25 cm depth from each block and a composite sample (approximately 30 kg) was compiled at each site. Composite soil was used for filling pots, one kg of soil was transferred to each pot in a completely randomised experiment with the following treatments (seven replications per treatment): 1) *P. patula* control planted in untreated old-field soil; 2) *P. patula* control planted in untreated virgin soil; 3) Weeds planted together with *P. patula* seedlings in old-field soil; 4) Weeds planted together with *P. patula* seedlings in virgin soil; 5) *P. patula* planted in steam pasteurized and metalaxyl treated old-field soil; 6) Weeds planted together with *P. patula* seedlings in steam pasteurized and metalaxyl treated old-field soil.

A seed mixture of three weed species *C. sumatrensis, B. pilosa* and *T. minuta* was planted at the rate of 0.5 g seeds per pot in treatment three, four and six. The foliage of weeds were trimmed back regularly to a height of 2-3 cm to minimize light competition among weed and pine seedling. Steam pasteurization of the soil was done at 80ºC for three hours. To inhibit *Pythium,* Metalaxyl (Ridomyl 10% WP Ciba-Geigy) was applied to the steam pasteurized soil in treatment five and six, as a soil drench at the rate of 1.6 g L^{-1} of soil. Nutrient supplement solution (Nitsch, 1972) was added once a week at the rate of 200 mL per pot. Plants were grown for five months in the greenhouse with an average daily temperature varying between 15.5 and 35ºC.

After harvest, growth parameters i.e. stem length, stem diameter, root and shoot dry weights were recorded. Composite soil samples from each treatment were analyzed for pH, P, Ca, Mg and K by the Department of Plant Production and Soil Science, University of Pretoria. Infection of roots by *Pythium* spp. was assessed by plating a total of 100 root pieces per treatment on a selective medium. Roots were cut into 5 mm long pieces, surface sterilized in 1.5% sodium hypochloride and plated on the medium of Mitchell and Kannwischer-Mitchell (1992) for detection of *Pythium* spp. Soil infestation by *Pythium* spp. was assessed by means of the citrus leaf disc method (Grimm & Alexander, 1973). EM colonization of roots was assessed and quantified under a dissecting microscope by recording the number of EM root tips per cm of the root on 150 root pieces per treatment.

The data were subjected to analysis of variance and treatment's mean differences were determined by DMR test using SAS procedures (SAS Institute, 1982).

RESULTS AND DISCUSSION

Soil analysis showed that pH of all soils except AO3 (old land) ranged between 5-5.1 (Table I). Soil phosphorus was low in soil from CO9 and C17/18, medium in AO3 old land and high in AO3 virgin land. Calcium was comparatively higher and magnesium lower in AO3 old land, which might be the reason for the higher pH.

Plant growth as indicated by stem diameter and shoot length was significantly greater (p <0.05) in pasteurized soil (Table II). Growth was poorest where weeds were grown along with the pine seedlings in either old land or virgin soils. Since direct competition for nutrients and light was minimized by supplying nutrient solution and cutting back weed foliage regularly, it is evident that weeds affected pine growth through some other mechanism. The effect of weeds on plant growth was similar in old land and virgin soil.

Pythium spp. occurred in both virgin and old land soil at all three sites assessed, with the incidence ranging from 19 to 23% (Table III). This corresponds with the findings of Linde *et al*. (1994) who also reported the occurrence of *Pythium* spp in both virgin and old land soils. The pine seedlings received from Mondi nursery and tested prior to initiation of this experiment showed infection in 40% of the seedlings. This finding explains the occurrence of low levels of *Pythium* spp. in the pasteurized soils (Table III). There was, however, a marked increase in the incidence of *Pythium* spp. in the pasteurized soils in the presence of weeds. *Pythium* spp. could not be isolated from the roots of pine grown in pasteurized old land soil without weed.

Table I. Chemical characteristics of the old land and virgin soils

Site*/Soil	pH (H_2O)	Bray P1 (mg kg^{-1})	Ammonium acetate extractable cations (mg kg^{-1})		
			Ca	Mg	K
CO9 OLS	5.0	2.81	406	172	338
CO9 VS	5.1	2.81	374	192	113
AO3 OLS	5.5	10.46	752	216	32
AO3 VS	5.1	32.41	410	94	61
C17/18 OLS	5.1	3.94	418	230	82
C17/18 VS	5.0	1.71	240	122	352

* Designation of site at Mondi estate, Kwazulu Natal; OLS = Old land soil; VS = Virgin soil

Table II. Shoot growth of *Pinus patula* seedlings in variously treated old land and virgin soils

Treatment	Shoot length (L) and diameter (D) mm					
	L	D	L	D	L	D
	CO9*		AO3*		C17/18*	
OLS (untreated)	92.8b*	8.1ab	393.6bc	8.1ab	462.8ab	7.8bc
VS (untreated)	320.0bc	6.4d	467.8ab	7.2bc	446.4ab	8.2bc
OLS + weeds	350.0bc	6.9Cd	305.7c	6.2cd	420.7b	7.2c
VS + weeds	383.6c	5.3e	352.8c	6.1d	396.4b	6.2d
OLS (treated)	544.3a	9.0a	536.4a	8.7a	522.1a	9.9a
OLS + weed (treated)	407.1b	7.5bc	349.2c	6.7cd	396.1b	8.7b

*Mean of seven replicates; Means followed by the same letter in the column are not significantly different at $P = 0.05$ (Duncan's multiple range test); * Designation of site at Mondi estate, Kwazulu Natal; OLS = Old land soil; VS = Virgin soil; Treated = Pasteurized and Metalaxyl treated soil

However, in similarly treated old land soil with weeds, *Pythium* spp. were isolated from 7.6, 8.5 and 4.7% of the roots from sites CO9, AO3, and C17/18, respectively. These findings indicate a possible predisposing effect of weeds on pine roots, as well as a stimulator effect on *Pythium* in the soil. No artificial EM inoculum was added during the present study, but plants were dependent on two possible sources of EM inoculum. Firstly, the nursery seedlings examined prior to initiation of this experiment had an average of 4.2 EM tips per cm of the root and the EM fungus present on the roots was identified as *Thelephora terrestris*. *T. terrestris* commonly occurs in pine nurseries in Kwazulu-Natal (Van Greuning & van der Westhiuzen, 1990) presumably originating from the pine bark in which pine seedlings are grown. Secondly, the soils used in the present study could have contained naturally occurring EM fungal inocula. From the data (Table IV), it is apparent that EM colonization decreased in the presence of weeds in all three soils (CO9, AO3 and C17/18), compared to the same treatments without weeds. This trend was however statistically significant only in pasteurized old land soils. The level of EM colonization was significantly higher in virgin as opposed to old land soil (in the absence of weeds) only in soil from site C17/18. The present data demonstrating an inhibitory effect of weeds on EM activity confirms previous reports of allelopathic effects of weeds on EM of black spruce (Mallik & Zhu, 1995) and conifers (Robinson, 1992).

Our data demonstrates the involvement of a complex of many causal factors in the old land syndrome of *P. patula*. Results indicate a tripartite interaction between EM fungi, *Pythium* and weed allelopathy, in which the presence of weeds weakens the pine by inhibiting EM fungal colonization as well as predisposing plants to *Pythium* infection. The possibilities of the alleviation of the old land syndrome by eradication of weeds and *Pythium* infestation as well as establishment of effective EM populations in newly afforested areas should be investigated.

Table III. Incidence of *Pythium* spp. in variously treated virgin and old land soils and in the roots of *Pinus patula* seedlings planted in these soils

Treatment	*Pythium* infestation (%)					
	Root	Soil	Root	Soil	Root	Soil
	CO9*		AO3*		C17/18*	
OLS (untreated)	0.00	19.23*	0.00	23.26	2.85	22.22
VS (untreated)	0.00	19.23	0.00	23.26	0.00	20.00
OLS + weeds	20.95	19.23	7.60	20.93	7.60	22.22
VS + weeds	20.00	17.31	20.90	23.26	12.38	17.78
OLS (treated)	0.00	9.62	0.00	2.33	0.00	2.22
OLS + weeds (treated)	7.60	15.38	8.50	6.98	4.76	15.56

*Mean of 100 observations; * Designation of site at Mondi estate, Kwazulu Natal; OLS = Old land soil; VS = Virgin soil; Treated = Pasteurized and Metalaxyl treated soil

Table IV. Ectomycorrhizal fungal colonization of *Pinus patula* in variously treated virgin and old land soils in the presence and absence of weeds

Treatment	Total EM fungal root tips cm^{-1} of root		
	CO9*	AO3*	C17/18*
OLS (untreated)	6.12ab*	4.79b	6.65c
VS (untreated)	5.99b	4.97b	10.95a
OLS + weeds	4.45bc	3.93b	5.65c
VS + weeds	6.29ab	4.91b	9.66a
OLS (treated)	7.34a	6.75a	9.00b
OLS + weeds (treated)	3.68c	4.74b	4.99c

*Mean of 150 observations; Means followed by the same letter in the column are not significantly different at $P = 0.05$ (DMR test);
* Designation of site at Mondi estate, Kwazulu Natal; OLS = Old land soil; VS = Virgin soil; Treated = Pasteurized and Metalaxyl treated soil

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