EFFECT OF MYCORRHIZAL FUNGI ON THE GROWTH AND NUTRIENT UPTAKE OF CARIBBEAN PINE SEEDLINGS

By

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Introduction

There is evidence (Hatch 1937; Lamb & Richards, 1971) that ectomycorrhizal fungi greatly enhance plant growth and nutrient uptake, and Harley (1969) reported that under certain conditions, inoculated plants grow better than the uninoculated controls. There are also reports of physiological differences between ectomycorrhizal fungi in promoting tree growth and improving nutrition (Bowen, 1968; Lamb & Richards, 1971) Thus good ectomycorrhizal development on the roots of tree seedlings is a *sine qua non* for seedling survival and growth.

Recent research (Lamb & Richards, 1970; Ekwebelam, 1977) has shown that there are mycorrhizal fungi capable of forming ectomycorrhizas which fruit rarely or not at all, and which therefore have neither been cultured nor identified. Previous reports (Lamb & Richards, 1971; Lamb, 1974) showed that in glasshouse or nursery experiments, these species stimulated growth and nutrient uptake of pine seedlings better than some named mycorrhizal fungi.

This paper presents further evidence of differences between ectomycorrhizal fungi, including unidentified species isolated from roots, in promoting growth and nutrient uptake of pine seedlings in glasshouse studies.

Material and methods

Soil sterilization :—The potting medium used was taken from the top 12 cm of the 'Glasshouse Sand' (Vallance, 1938). The soil is extremely deficient in plant nutrients, particularly phosphorus (see Appendix 1). The soil was sterilized for 45 min. at 103 kN/m^2 and then put in 70 sterile 10 cm diameter plastic pots (575 gm/pot), and watered with de-ionized water a day prior to transplanting and inoculation of seedlings.

Seed sterilization :—Seeds of Carribean pine (*Pinus caribaea* var. *hondurensis* Barr. & Golf.) were surface-sterilized as previously described (see Ekwebelam, 1977) and aseptically planted on sterile nutrient agar, and incubated at 25$\pm$1° C in the dark. All contaminated seeds were discarded.

Inoculum production :—The fungi used were 4 known mycorrhizal species [*Pisolithus tinctorius* (Pers.) Coker & Couch; *Rhizopogon luteolus* Fr. & Nordh; *R. roseolus* (Corda) T.M. Fr. and *Suillus granulatus* (L. ex Fr.) O. Kuntze] and 8 unidentified basidiomycete species isolated from mycorrhizal roots of pines (coded M 1—M 8) as previously described (Ekwebelam, 1977). The culture of *Pisolithus*, *Suillus* and *R. luteolus* were provided by Dr. R.J. Lamb Department of Environmental Biology, School of Life Sciences, New South Wales Institute of Technology, Gore Hill, NSW; Australia. The isolate of *R. roseolus* came from Dr. E J. Hacskaylo of the Pathology Division, USDA Forest Service, Beltsville, Md. Pure cultures were grown in bulk on sterile modified Hagem agar (Modess, 1941) at 22 ± 1°C.

Planting and Inoculation :—Planting and inoculation were done simultaneously. Replicate pots each containing 2 seedlings were aseptically inoculated with agar discs (2 cm diameter) five for each fungus. Pots were then placed on a wooden bench in a glasshouse in a randomised block design; the controls duplicated. The pots were watered with de-ionized water, and basal dressings of $NaH_2PO_4 . 2H_2O$ at the rate of 10 ml/pot of 50 ppm P solution (i.e. 0.5 mg P/pot) were applied at intervals of 10, 13 and 17 weeks from planting. Plant height in centimeters taken from the cotyledonary scars to shoot apices (see McComb, 1943) was first measured at 2 months and at monthly intervals thereafter, and the experiment was terminated at 6 months.

Harvest :—At harvest, roots were cleansed of soil particles with running tap water and examined for the presence of mycorrhizas. Reisolations and confirmation of identity of original fungi used for inoculation were effected as previously described by Ekwebelam (1977). Mycorrhizal infection per seedling was assessed by the percentage mycorrhiza as elaborated by Richards & Wilson (1963). Whole plants dried in a mechanical convection oven at 80°C for 48 hr. weighed and ground in a 'Casella' grain mill to pass through a 1 mm sieve were used for the determination of total NPK. Nitrogen was determined by the modified micro-kjedahl method (Bremner, 1965). and after dry ashing, phosphorus and potassium were measured using Truog & Meyer (1929) phospho-molydate blue for P, and a flame photometric method for K.

Results

Height development :—Mean seedling height over the 6 months from planting (Table 1) show that a growth differential was already apparent 2 months from planting, and although there were consistent improvements in growth following inoculation with fungi, these were statistically non-significant. However, inoculated plants generally grow better than the uninoculated controls, with *S. granulatus* better than with *R. roseolus* and the unidentified M4, and with *P. tinctorius* the poorest. M2 and M1 stimulated growth early but this was not sustained. This observantion supports the view that mycorrhizas differ in their effectiveness and some are more effective than others in promoting tree growth (Harley, 1969; Lamb & Richards, 1971).

Mycorrhizal status :—There were significant differences at the 5% probability level (Duncan's Multiple Range Test) in the proportion of short roots converted to mycorrhizas by M1, M4 and M5 (Table 2) suggesting that treatment effects were related to mycorrhizal infection. *R. roseolus* was also isolated from some of these mycorrhizas, and from the 2 uninoculated controls which may account for the differences between them.

Dry matter content :—There were significant differences at the 5% probability level (Duncan's Multiple Range Test) between inoculated and uninoculated seedlings, and between the different fungal species (Table 2). Inoculation with M4 and *R. roseolus* gave the highest, and with *P. tinctorius* the lowest dry matter.

Nutrient concentration :—Differences were evident at the 5% probability level (Duncan's Multiple Range Test) in the concentrations of nitrogen and potassium among the different treatments (Table 3). Inoculation with M4 resulted in plants with low N and whereas M5, M7 and *P. tinctorius* increased the K concentration, M2 decreased it. Regression analyses indicated that N (r^2 = 0.05) and K (r^2 = 0 15) concentrations were independent of the intensity of mycorrhizal infection.

Higher significant differences occurred with respect to P concentration of plants inoculated with M1, M2 and M5. M1 and M5 were among those that produced largest numbers of mycorrhizas, thus supporting the widely held premise that plants with the highest levels of mycorrhizal infection show the greatest absorption of P (Harley 1969) Regression analysis indicated that numbers of mycorrhizas were correlated with P concentration (r^2=0.48).

Nutrient content :—This reflects the combined influences of the different inoculum species on dry matter content and nutrient concentrations (Table 3). There were significant differences at the 5% probability level (Duncan's Multiple Range Test) in the uptake of N, P, K, between inoculated and uninoculated seedlings, the best causing five-fold increase in the uptake of P over the better of the controls. *R roseolus* was the only fungus to increase the uptake of N. M1, M2 and M5 proved most effective in P uptake, and M5, M4 and M6 gave a greater stimulus to K uptake, than did any of the other species. Regression analyses indicated that numbers of mycorrhizas (i e % roots) were correlated with the uptake of P (r^2 = 0.59) and K (r^2 = 0.30), but not with the uptake of N (r^2 = 0 14).

Table 1

Mean heights (cm) of pine seedlings in response to inoculation with named and unnamed mycorrhizal fungi

Inoculum (Name or code number)	Months from planting 2	3	4	5	6
	Height (cm)*				
Control (uninoculated)	1.3	1.6	1 9	2.1	2.2
M1	1.7	2.1	2 7	2 9	3.0
M2	1.4	2 2	2.7	3 0	3.0
M3	1.3	1.7	2.1	2 6	3 0
M4	1.4	1 8	2 3	2 7	3.1
M5	1.3	2.0	2.4	2.7	2.9
M6	1.5	1 9	2 3	2 7	2.8
M7	1.2	1.6	2 0	2.4	2.6
M8	1.5	2.0	2.4	2.6	2.8
Pisolithus tinctorius	1.2	1.4	1.8	2.0	2.1
Rhizopogon luteolus	1.6	2.0	2.3	2 7	3 0
R. roseolus	1.5	1.9	2 2	2.8	3.3
Suillus granulatus	2.0	2.2	3.1	3.5	4 0
Control (uninoculated)	1.1	1.3	1.5	1.9	2.0
LSD (P<0 01)	0.8	0.9	1.2	1.3	1.4
LSD (P<0.05)	0.6	0.7	0.9	1.0	1.1

*All figures are means of 5 replicates, measuring from cotyledonary scars to shoot apices.

Table 2

Effect of inoculation with different mycorrhizal fungi on mycorrhizal infection and dry matter production of pine seedlings (All figures are means of 5 replicates)

Inoculum (Name or code number)	Mycorrhiza[1]	Mycorrhiza (%)[1]	Dry matter (g)
Control (uninoculated)	+	9.4g[2]	2.1de
M1	+	82.3ab	2.5cde
M2	+	57.6bcd	2.6bcde
M3	+	22.4efg	2.4cde
M4	+	64.3abc	4.0a
M5	+	91.7a	2.6bcde
M6	+	48.4cde	3.5abc
M7	+	47.8cde	2.5cde
M8	+	29.4def	3.2abcd
Pisolithus tinctorius	+	38.9cdef	2.2cde
Rhizopogon luteolus	+	45.6cde	3.4abcd
R. roseolus	+	39.5cdef	3.9ab
Suillus granulatus	+	56.[illegible]bcd	3.5abc
Control (uninoculated)	+	12.3fg	1.7e

[1] Mycorrhiza present (+) or absent (—). Proportion of short roots converted to mycorrhizas.

[2] In each parameter, means followed by the same lower case letter are not significantly different at the 5 per cent probability level (Duncan's Multiple Range Test).

Table 3

Effect of inoculation with different mycorrhizal fungi on nutrient status of pine seedlings (All figures are means of 5 replicates)

Inoculum (Name or code number)	Concentration (%)			Content (mg)		
	N	P	K	N	P	K
Control (uninoculated)	0.322bc	0.010e	0.400d	6.83c	0.2[illegible]d	8.48c
M1	0.46[illegible]ab	0.048a	0.604abcd	11.29b	1.18a	14.80abc
M2	0.451ab	0.046a	0.464d	11.90b	1.21a	12.25bc
M3	0.461ab	0.017cde	0.628abcd	10.93b	0.40c	14.88abc
M4	0.285c	0.014de	0.548cd	11.31b	0.56c	21.76a
M5	0.389abc	0.038b	0.876a	10.19b	1.00ab	22.95a
M6	0.353abc	0.015cde	0.616abcd	12.32b	0.52c	21.50a
M7	0.438ab	0.020cd	0.836ab	10.95b	0.50c	20.90ab
M8	0.359abc	0.020cd	0.616abcd	11.45b	0.64c	19.65ab
Pisolithus tinctorius	0.486a	0.024c	0.784abc	10.84b	0.54c	17.48ab
Rhizopogon luteolus	0.334bc	0.019cd	0.548cd	11.22b	0.64c	18.41ab
R. roseolus	0.394abc	0.021cd	0.532cd	15.25a	0.81bc	20.59ab
Suillus granulatus	0.357abc	0.023cd	0.588bcd	12.64b	0.81bc	20.82ab
Control (uninoculated)	0.346abc	0.014de	0.450d	5.99c	0.24d	7.79c

[1] In each parameter, means followed by the same lowercase letter are not significantly different at the 5 per cent probability level (Duncan's Multiple Range Test).

Appendix 1

Chemical data of Glasshouse Sand (After Vallance, 1978)

Composition %	0—10	Depth in cm 10—38	38—61	61—193
R_2O_3*	2 630	4.310	12 220	16.120
F_2O_3	1.260	1.920	3 860	6 000
P_2O_5	0 013	0.021	0 020	0 018
CaO	0 056	0.028	0.028	0 035
MgO	0.014	0 036	0.004	0 051
K_2O	0.270	0.023	0 023	0.042
Organic C	1.300	0.510	0.520	0.180
pH	6.0	5.8	6 0	5 8

*R_2O_3 refers to iron and aluminium sesquioxides

Discussion

Inoculation with mycorrhizal fungi, including unidentified species isolated from mycorrhizal roots of pines in the glasshouse can stimulate plant growth and nutrient uptake, the magnitude of the effect differing fungi. Other workers (Harley, 1969; Theodorou & Bowen, 1970; Lamb & Richards, 1971) have reached similar conclusions. Although the reasons for the differing effectiveness of various fungi remain unknown, Hatch (1937) and Harley (1969) have suggested that the continued survival of conifer seedlings in an area is dependent on the presence of mycorrhizas as organs for absorption and translocation.

Fungi differ markedly in their ability to form mycorrhizas and Melin (1925) classified this in terms of activity' and virulence' of the fungus. The former denotes inherent ability of a fungi to form mycorrhizas with the host whose roots it contacts, and the latter the ability of the fungus to produce auxin in concentration that will ensure the degree of morphogenesis necessary for the establishment of mycorrhizal symbiosis Slankis, 1958). These phenomena can be altered by extraneous factors such as soil characteristics host physiology and temperature (Mejstrik, 1970). These characters influence the effects of the fungus on the host, and the balance or lack of it in the symbiotic relationship (Trappe, 1962). Since Melin's observation of these phenomena, occasional references on the effects of nutritional factors and other internal variables influencing physiological differences among fungi, have been made (see Harley, 1969; Bowen & Theodorou, 1967). Which of these possible mechanisms was operative in influencing the results of the present study is not known.

Judging from the results of the data in Table 1—3 some of the unidentified fungi appeared better than some recognised mycorrhizal species in their effects on mycorrhizal development, dry matter production and nutrient status of the host seedlings. It is interesting to note that although these fungi were isolated directly from mycorrhizal roots of pines, their identity could not be established even when compared with pure cultures of sporophores of suspected mycorrhiza formers from the same area (see Ekwebelam, 1977). These results supported by those of Lamb & Richards (1970, 1971) and Ekwebelam (1977) confirm that much still remains to be learnt of the identity and relative effectiveness of the various mycorrhizal endophytes of pines, and open a new vista for research on the biology and autecology of these 'lesser' know mycorrhizal symbionts.

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SUMMARY

Twelve ectomycorrhizal fungi (*Pisolithus tinctorius*, *Rhizopogon luteolus*, *R. roseolus*, *Suillus granulatus* and 8 unidentified species isolated from Caribbean pine) were compared for their effectiveness in stimulating growth and nutrient uptake in glasshouse grown seedlings of Caribbean pine. Differences occurred between the different fungi in their stimulation of growth and nutrient status of host seedlings. Inoculation with *S. granulatus* and *R. roseolus* gave the best stimulation of height growth, and *P. tinctorious* the poorest. Some unidentified fungi appeared superior to the known mycorrhizal species as shown by mycorrhizal infection, dry matter production, and the quantity of phosphorus and potassium absorbed. The number of mycorrhizas produced was correlated with the concentration of P, and the total quantity of P and K absorbed.

कैरिबियाई चीड़ के पौधों की वृद्धि और पोष्याहार उद्ग्रहण पर मूलसहजीवी कवकों का प्रभाव

लेखक एस० ए० एकवेबेलम

सारांश

बारह बाह्य मूल सहजीवी कवकों (*पिसोलिथस टिंक्टोरियस, राइजोपोगोन ल्यूटियोलस*, रा० *रोजियोलस*, *सुइलस ग्रैनुलेटस* तथा कैरिबियाई चीड़ से पृथक की गई आठ अन्य अनपहचानी जातियां) की कैरिबियाई चीड़ के कांच घर में उगाए पौधों में वृद्धि तथा खनिज उद्ग्रहण प्रोत्साहित करने के लिए तुलना की गई। पोषी पौधों की वृद्धि तथा पोष्याहार स्थिति में विभिन्न कवकों में अन्तर पाया गया। सु० *ग्रैनुलेटस* और रा० *रोजियलस* का टीका लगाने से ऊंचाई बढ़ने में सर्वाधिक प्रेरणा मिली। पि० *टिंक्टोरियम* से सबसे कम। कुछ अनपहचाने कवक जाने हुए कवकों की अपेक्षा श्रेष्ठतर दिखाई पड़े जैसा कि कवकमूलता संक्रमण, शुष्क तत्व उत्पादन, प्रचूषित फास्फोरस और पोटाशियम मात्रा से मालूम हुआ। उत्पादित कवकमूलता संख्या का सहसम्बन्ध फास्फोरस संकेन्द्रण, प्रचूषित फास्फोरस और पोटाशियम की कुल मात्रा से दिखलाया गया।

Wirkung der mycorrhizalen Giftpilzen am Wuchs und am Nahrungbegreifen bei Carribaen Fichte Sämlingen

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ZUSAMMENFASSUNG

Zwölf ectomycorrhizalen Giftpilzen (*Pisolithus tinctorius*, *Rhizopogon luteolus*, *R. roseolus*, *Suillus granulatus* und 8 unidentifizierten Arten, von Carribaen Fichte isoliert) waren um ihrer Wirksamlichkeit das Wachstum und Nahrungbegreifen bei den treibhausgewachsenen Sämlingen der Carribaen Fichte zu ansparen, verglichen. Der Unterschied kommt zwischen verschiedenen Giftpilzen, um seiner Anregung das Wachstum und nahrunge Stellung der gastgebere Pflanzen vor. Einimpfung mit *S. granulatus* und *R roseolus* gab die besten Anregung das Höhewachstums, und mit *P. tinctorius* die schlechtesten. Einigen unidentifizierten Giftpilzen auch erscheinten über den genannten mycorrhizalen Arten besser, als das bei der mycorrhizale Ansteckung, trockene stoffe Produktion and die Quantität der Absorption des Phosphors und des Kaliums angezeigt war. Die Zahl der produzierten Mycorrhizalen war zur Konzentration des P, der ganze eingesaugten Quantität der P und K, verwandt.

Effets de Champignons mycorrhizes sur la croissance et l'absorption de matière nutritive chez les semis de "Carribbean Pine".

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Résumé

Cette étude porte sur douze copèces des Champignons mycorrhizes. (*Pisolithus tinctorius*, *Rhizopogon luteolus*, *R. roseolus*, *Suillus granulatus* et 8 espèces non identifiées, isolées de "Carribbean Pine" et on les a comparées à l'égard de leur efficacité pour activer la croissance et l'absorption de matière nutritive chez les semis de "Carribbean Pine", élevés dans une chambre à verve. Les divers Champignons ont fait voir des différences entre eux, à l'egard de l'activation de croissance et l'absorption de matière nutritive. L'inoculation avec *S. granulatus* et *R. roseolus* a activé au maximum et celle avec *P. tinctorius* le minimum, la croissance en hauteur. Quelques champignons non identifiés se sont montrés supériour aux espèces mycorrhizes identifiés, ce qui est manifesté par l'attaque des Champignons mycorrhizes, la production de la matière sèche et la quantité de phospore et potassium absorbée. La nombre de mycorrhizes produits était en correlation avec la concentration de P et la quantité totale de P et K absorbée.

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