EFFECT OF WATERLOGGING ON MYCORRHIZAS OF RADIATA PINE AND DOUGLAS FIR

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ABSTRACT

Radiata pine (**Pinus radiata** D. Don) and Douglas fir (**Pseudotsuga menziesii** (Mirb.) Franco.) seedlings were subjected to waterlogging periods of 1, 2, 4, 8, and 16 weeks. Phosphorus-32 uptake and succinic dehydrogenase activity of waterlogged mycorrhizas were measured and compared with non-waterlogged mycorrhizas. After 2 weeks of waterlogging Douglas fir mycorrhizas absorbed less ^{32}P than control mycorrhizas; radiata pine mycorrhizas were not significantly affected. Waterlogging periods of 4 to 16 weeks reduced ^{32}P uptake and succinic dehydrogenase activity in both species.

INTRODUCTION

Conifers with mycorrhizal root systems generally make much better growth in the field than non-mycorrhizal plants (White, 1941; Mishustin, 1967; Wilde, 1968) and a knowledge of the factors that affect mycorrhizas adversely is important. Gilmour (1958) found that Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco.) from Milton Nursery showed severe chlorosis and poor growth in the field. This condition was shown to be associated with lack of mycorrhizas. He noticed that mycorrhizal development in the nursery was very poor and suggested that this was due to the seasonal waterlogging which occurred there. Thus there is some evidence that mycorrhizas are adversely affected by waterlogging. This study was undertaken to find out the effect of various periods of waterlogging on mycorrhizas of two important forest species in New Zealand —radiata pine (*Pinus radiata* D. Don) and Douglas fir.

According to Gill (1970), in previous work on the effect of waterlogging on tree roots, root vitality appears to have been judged qualitatively. Since it is very difficult to decide whether a root is dead, half-dead or alive, an attempt was made in this study to make an objective quantitative measurement of root vitality. In the presence of living tissue, colourless tetrazolium salts are reduced to coloured formazan and it has been shown that dehydrogenases are responsible for this reduction (Mattson *et. al.,* 1947). Succinic dehydrogenase activity has been demonstrated using tetrazolium salts (Nachlas *et al.,* 1957; Avers and King, 1960; Morrison and Kronheim, 1962). Hauser and Morrison (1964) showed that viable and non-viable pollen grains could be distinguished using succinic dehydrogenase activity (SDA) as the criterion of viability. Defendi and Pearson (1955) made a detailed study of the quantitative estimation of SDA and showed that 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride could be used reliably for estimating the amount of succinic dehydrogenase present in

the tissues. In this study SDA as estimated by a method similar to that of Defendi and Pearson was used as a measure of the vitality of mycorrhizas.

It has been established that the rate of uptake of phosphate by mycorrhizal roots is greater than that by non-mycorrhizal roots in radiata pine (Morrison, 1957; Mejstrik, 1970) and it has been suggested that the same is true for roots of Douglas fir (McComb and Griffiths, 1946). Plants with well developed mycorrhizas would be expected to absorb more phosphate than plants with poor mycorrhizas and in this study the effectiveness of mycorrhizas was estimated by measuring phosphorus-32 (^{32}P) uptake.

MATERIAL

Seedlings of radiata pine (1 yr-old) and Douglas fir (2 yr-old) were selected in the nursery for uniform size and visually for profuse mycorrhizal development. The selected seedlings were potted, some in unsterilised nursery soil and some in unsterilised sand from Woodhill State Forest, in 150-mm pots. After potting, the seedlings were kept in a glasshouse at 15° ± 2°C for 4 months to allow them to recover from transplanting shock and to make new root growth. The seedlings potted in soil were used for estimation of SDA. Uptake of ^{32}P was estimated using seedlings potted in easily draining sand to ensure that, after the waterlogging period was terminated, phosphate uptake by the mycorrhizas was not reduced by lack of aeration in the pots which had been waterlogged (Harley *et al.*, 1953).

Thirty seedlings (15 in soil and 15 in sand) of each species were placed in polyethylene-lined irrigation trays which were filled with water so that the pots were just submerged. An equal number of seedlings were kept as controls and watered normally. Six seedlings (three in soil and three in sand) were removed from the trays at intervals of 1, 2, 4, 8, and 16 wk. They were allowed to drain overnight. Next day, the three seedlings from waterlogged soil and three control seedlings (also in soil) were used for estimation of SDA. The three seedlings from waterlogged sand and three control seedlings (in sand) were used for measurement of ^{32}P uptake.

METHODS

1. Estimation of SDA

Seedlings were removed from the pots and the roots were carefully washed under a tap. Ten mycorrhizal clusters (dichotomously branched short roots with fungal mantle in radiata pine and clusters of the pinnate type in Douglas fir) about 0.5 ml in volume, were selected from each pot. Five of these came from near the top of the pot and five from near the bottom. The volume of each mycorrhizal cluster was determined using a modification of the volumeter described by Clark (1961). Roots were stored separately at —16°C for 16 hr and then each root was chopped up in 6 ml of incubating solution made up as follows: 2 ml of 0.1 M phosphate buffer at pH 7.4 + 2 ml of 0.1 M sodium succinate + 2 ml of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (1 mg/ml). The macerated tissue and the incubating solution mixture were placed in stoppered vials and incubated at 25°C for 3 hr (Burrows and Carr, 1969). After incubation the mycorrhizas were dried by placing the unstoppered vials in a forced draught oven at 45°C. The reduced tetrazolium salt was extracted in 5 ml ethyl acetate and the amount of the reduced salt in ethyl acetate was read in a spectrophotometer at

494 nm. In a preliminary experiment using healthy mycorrhizas it was found that the amount of the reduced tetrazolium salt was directly proportional to the volume of the mycorrhizal cluster (correlation coefficients: radiata pine = +0.938; Douglas fir = + 0.978). For each set of determinations controls of substrate incubated without roots were run. In no case was reduction of the tetrazolium salt observed in the controls. Results were calculated in terms of absorbance per unit volume of mycorrhizal cluster.

2. Uptake of ^{32}P

The waterlogged pots were allowed to drain overnight; this brought the sand to field capacity. In the form of orthophosphate ^{32}P was applied in solution (300 ml/pot; specific activity 0.51 - 0.59 μc/ml) evenly over the surface of the pots and the pots were allowed to drain. Since 300 ml of water were required to bring the sand in one pot to field capacity, it is assumed that the orthophosphate solution displaced the water already present in the pots. After 16 hr the plants were removed from the pots and washed thoroughly in eight changes of tap water and three changes of distilled water. The plants were divided into roots and shoots and dried to constant weight at 80°C in a forced draught oven. The plant material was then ground and four 0.1 g subsamples were taken from each sample. These were measured for radioactivity in a gas-flow counter with end window and the resultant data corrected for radioactive decay, for variations in counter efficiency, and for background.

RESULTS AND DISCUSSION

Visual observations on root systems of both species showed that the control seedlings had large numbers of mycorrhizas, white root tips, and light brown suberised roots. Waterlogging for 1 wk caused no discernible change. After waterlogging for 2 wk, the young root tips had turned a purplish black but the mycorrhizas appeared normal. After waterlogging for 4 wk or longer, the root tips were black, the mycorrhizas brown, and the roots dark brown.

A summary of the results of the estimations of SDA and ^{32}P uptake is given in Table 1. A waterlogging period of 1 wk had no significant effect on ^{32}P uptake and SDA of the mycorrhizas of either species. After 2 wk of waterlogging Douglas fir roots absorbed significantly less ^{32}P than the roots of control seedlings but SDA was not affected. This period of waterlogging had no significant effect on radiata pine roots as

TABLE 1—Uptake of ^{32}P, and succinic dehydrogenase activity (SDA) of waterlogged roots as a percentage of values obtained for non-waterlogged roots (Control for each waterlogging period = 100)

Waterlogging period (wk)	Radiata Pine		Douglas Fir	
	^{32}P uptake	SDA	^{32}P uptake	SDA
1	75.0	104.8	84.1	86.0
2	73.4	99.1	43.0*	89.9
4	51.0*	50.9**	33.0*	42.0**
8	62.5*	45.8**	63.1*	25.5**
16	39.6*	31.6**	16.5*	15.2**

* Significantly lower than control ($p < 0.05$)

** Highly significantly lower than control ($p < 0.01$)

evaluated by these two tests. Waterlogging periods of 4 wk or longer significantly reduced ^{32}P uptake and SDA in both species.

Dead roots continue to absorb water (Kramer, 1933) and it is possible that succinic dehydrogenase remained active for some time after the cells producing it had died. It is therefore likely that the vitality of mycorrhizas was overestimated by the two criteria used here. There was a sharp drop in the SDA of mycorrhizas between 2 and 4 wk of waterlogging, the SDA after 4 wk being about half of that after 2 wk. After 4 wk the drop in SDA slowed down and by the end of 16 wk radiata pine mycorrhizas showed about a third and Douglas fir mycorrhizas about a sixth of the activity of non-waterlogged mycorrhizas. Uptake of ^{32}P showed a similar trend except that there was an inexplicable rise after 8 wk of waterlogging. The reduction in SDA and ^{32}P uptake could be due to the gradual death of the fungal mantle and the cortex while the stele probably remained alive. It was not possible to study localisation of SDA by examining thin sections microscopically, as Defendi and Pearson (1955) did with liver and kidney tissue, because the colour imparted to the tissues by deposition of formazan was not deep enough to be clearly detectable. Newhook (1959) found that when radiata pine seedlings were waterlogged for 5 months, all roots appeared dead, but when normal watering was resumed after the waterlogging period, regeneration of roots occurred showing that the stele was not dead.

The results show that Douglas fir is more susceptible to waterlogging than radiata pine. The results, however, do not agree with those of Minore (1968) who reported that the entire root system of Douglas fir seedlings was dead after 4 wk waterlogging. In the present experiment, although continued waterlogging lowered ^{32}P uptake and SDA of roots, even after 16 wk the roots tested were not entirely dead.

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REFERENCES

AVERS, C. J., and KING, E. E. 1960: Histochemical evidence of intracellular enzymatic heterogeneity of plant mitachondria. **American Journal of Botany 47:** 220-5.

BURROWS, W. J., and CARR, D. J. 1969: Effects of flooding the root system of sunflower plants on the cytokinin content in the xylem sap. **Physiologia Plantarum 22:** 1105-12.

CLARK, J. 1961: Photosynthesis and respiration in white spruce and balsam fir. **State University College of Forestry, Syracuse University, New York, Technical Paper 85.**

DEFENDI, V., and PEARSON, B. 1955: Quantitative estimation of succinic dehydrogenase activity in a single microscopic tissue section. **Journal of Histochemistry and Cytochemistry 3:** 61-9.

GILL, C. J. 1970: The flooding tolerance of woody species—a review. **Forestry Abstracts 31:** 671-88.

GILMOUR, J. W. 1958: Chlorosis of Douglas fir. **New Zealand Journal of Forestry 7:** 94-106.

HARLEY, J. L.; McCREADY, C. C., and BRIERLEY, J. K. 1953: The uptake of phosphate by excised mycorrhizal roots of beech IV. The effect of oxygen concentration upon host and fungus. **New Phytologist 52:** 124-32.

HAUSER, E. J. P., and MORRISON, J. H. 1964: The cytochemical reduction of nitro blue tetrazolium as an index of pollen viability. **American Journal of Botany 51:** 748-52.

KRAMER, P. J. 1933: The intake of water through dead root systems and its relation to the problem of absorption by transpiring plants. **American Journal of Botany 20:** 481-92.

MATTSON, A. M.; JENSEN, C. O., and DUTCHER, R. A. 1947: Triphenyltetrazolium chloride as a dye for vital tissues. **Science 106:** 294-95.

McCOMB, A. L., and GRIFFITH, J. E. 1946: Growth stimulation and phosphorus absorption of mycorrhizal and non-mycorrhizal northern white pine and Douglas fir seedlings in relation to fertiliser treatment. **Plant Physiology 21:** 11-7.

MEJSTRIK, V. 1970. The uptake of ^{32}P by different kinds of ectotrophic mycorrhiza of **Pinus. New Phytologist 69:** 295-8.

MINORE, D. 1968: Effects of artificial flooding on seedling survival and growth of six northwestern tree species. **Pacific North West Forest and Range Experiment Station Research Note PNW 92.**

MISHUSTIN, E. N. 1967: Mycotrophy in trees and its value in silviculture. Pp. 17-34 **in** "Mycotrophy in Plants" (A. A. Imshenetskii, Ed.), Israel Program for Scientific Translations, Jerusalem.

MORRISON, T. M. 1957: Mycorrhiza and phosphorus uptake. **Nature, London 179:** 907.

MORRISON, J. H., and KRONHEIM, S. 1962: The cytochemical demonstration of succinic dehydrogenase in mouse leucocytes. **Journal of Histochemistry and Cytochemistry 10:** 402-11.

NACHLAS, M. M.; TSOU, K.-C.; de SOUZA, E.; CHENG, C.-S., and SELIGMAN, A. M. 1957: Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitropenhyl substituted ditetrazole. **Journal of Histochemistry and Cytochemistry 5:** 420-36.

NEWHOOK, F. J. 1959: The association of **Phytophthora** spp. with mortality of **Pinus radiata** and other conifers I. Symptoms and epidemiology in shelterbelts. **New Zealand Journal of Agricultural Research 2:** 808-43.

WHITE, D. P. 1941: Prairie soil as a medium for tree growth. **Ecology 22:** 398-407.

WILDE, S. A. 1968: Mycorrhiza: their role in tree nutrition and timber production. **College of Agriculture and Life Sciences, University of Wisconsin Research Bulletin 272.**