New Phytol. (1990), **115**, 311–317

Occurrence of cluster roots on *Myrica cerifera* L. (Myricaceae) in water culture in relation to phosphorus nutrition

By ISABELLE LOUIS, SUZANNE RACETTE and JOHN G. TORREY

Harvard Forest, Harvard University, Petersham, Massachusetts 01366, USA

(*Received 22 November 1989; accepted 1 February 1990*)

SUMMARY

When *Myrica cerifera* was grown in a number of $\frac{1}{4}$-strength Hoagland's solutions, each lacking one mineral nutrient, cluster roots formed only in solutions lacking phosphorus (P). In seedlings initially fertilized with various P concentrations, cluster root formation decreased with increasing P supply and was totally suppressed in solutions containing $\geqslant 1$ mg P l^{-1}. As total root weight did not vary significantly between P treatments, the proportion of the root system expressed as cluster roots or non-cluster roots varied with phosphorus supply. A similar response to increasing levels of foliar-applied P suggests that it may be internal P concentration in the plant that determines the initiation of cluster roots and not external P levels in the soil. Nitrogen, supplied as fixed dinitrogen or nitrate, did not have any effect on development of cluster roots and it appears that the capacity of actinorhizal plants to fix nitrogen by symbiotic association with a soil micro-organism is of no significance to cluster root formation. While abundant root hair development and extensive mycorrhizal mycelium distribution in the rhizosphere may represent more efficient morphological forms of improving plant P uptake, the significance of cluster roots in relation to P nutrition and the mechanisms involved in their development warrant further investigation.

Key words: Cluster roots, proteoid roots, *Myrica cerifera*, mineral deficiency, phosphorus, water culture.

INTRODUCTION

Dense clusters of rootlets which are produced periodically along elongating lateral roots were first described as proteoid roots for the family Proteaceae (Purnell, 1960; Lamont, 1972*a*, 1983). They were suggested to have evolved in response to the poor soil conditions where the Proteaceae typically occur (Jeffrey, 1967; Lamont, 1972*b*). Analogous structures have, however, also been reported on legumes, including *Viminaria juncea* (Lamont, 1972*c*; Walker & Pate, 1986), *Lupinus cosentenii* (Trinick, 1977) and *Lupinus albus* (Gardner, Parberry & Barber, 1981), and on some *Casuarina* species (Redell, 1986). Recognizing their more frequent occurrence beyond the Proteaceae, Lamont (1982) referred to these plants as 'cluster-rooted' plants. Cluster root formation has been demonstrated to be elicited by low phosphorus levels for some of the Proteaceae and legumes; the aggregation of rootlets had been suggested to enhance the plant's ability to retain and absorb phosphorus in nutrient poor soils (Jeffrey, 1967; Malajczuk & Bowen, 1974; Pathmaranee, 1974; Gardner, Parberry & Barber, 1982; Walker & Pate, 1986).

We have observed cluster root formation in several species of nitrogen-fixing actinorhizal plants within the Betulaceae, Casuarinaceae and Myricaceae, both in field and greenhouse studies. The objective of this investigation was to determine the influence of phosphorus on cluster root formation on *Myrica cerifera*, the common wax-myrtle, under controlled conditions in the greenhouse.

MATERIALS AND METHODS

Seedling preparation

M. cerifera L. (Myricaceae) is an evergreen which grows on sandy plains in southern and eastern USA. *M. cerifera* seeds (collected in Florida and kindly provided by Dr D. Baker) were germinated on moist sand for 2 months in an environmental growth chamber (maximum day temperature of 26 °C and night temperature of 18 °C; 16 h daylight). Seedlings

were watered daily with deionised water and fertilised three times weekly with modified $\frac{1}{4}$-strength Hoagland's solution (Hoagland & Arnon, 1950); after 2 months, each plant was carefully freed from sand and the roots washed in deionized water.

Growth conditions

To study cluster root formation in relation to phosphorus nutrition, seedlings were grown in $\frac{1}{2}$- or 1 l water culture jars (3 plants per jar), utilizing modified $\frac{1}{4}$-strength Hoagland's solution as substrate. The composition of this solution, in mg l^{-1}, was KNO_3 (126·25), $MgSO_4.7H_2O$ (123), $Ca(NO_3)_2.4H_2O$ (295), KH_2PO_4 (34), H_3BO_3 (0·715), $MnCl_2.4H_2O$ (0·4525), $ZnSO_4.7H_2O$ (0·055), $CuSO_4.5H_2O$ (0·02), $Na_2MoO_4.2H_2O$ (0·0062), $CoCl_2.6H_2O$ (0·0062), $FeSO_4.7H_2O$ (6·95), Na_2EDTA (9·31). In solutions lacking P, KH_2PO_4 was replaced with KCl (18·75 mg l^{-1}). Since preliminary studies had shown aeration to have a stimulatory effect on cluster root formation, all the jars were continuously aerated by bubbling air through 0·1 cm diameter plastic tubing. Experiments were conducted in the greenhouse with an average daily temperature range of 24–30 °C and 16 h daylight.

Experimental procedures

Experiment 1. This experiment set out to determine if any major mineral nutrients preferentially promoted cluster root formation. Seedlings were transferred to 1 l jars and grown in $\frac{1}{4}$-strength Hoagland's solution, either as a complete nutrient solution or lacking one of the following: Ca, Mg, Fe, S, K, P, N or micronutrients; the pH was 6·0 and 2 jars (6 plants) were established for each deficiency treatment. Deficiency series were set up as described in Machlis & Torrey (1956). The nutrient solutions were not renewed throughout the experiment but jars were topped up daily with deionized water. The ratio of the root volume to the water culture solution volume was such as to obviate serious deficiencies of inorganic ions other than the one omitted from the solution during the experimental period. The plants were harvested at 5 weeks.

Experiment 2. This study sought to investigate plant growth and onset of cluster root formation on *M. cerifera* growing in water culture prepared with a range of initial phosphorus concentrations. Seedlings were grown in $\frac{1}{4}$-strength Hoagland's solution in 1 l jars, with P supplied as KH_2PO_4 at levels of 0, 0·25, 0·5, and 1 mg l^{-1}. Three jars (9 plants) were established for each P level. The nutrient solutions were not changed over the 3-week period but topped up daily with deionized water. To determine the P levels that induced or suppressed cluster root formation, 15 ml of the nutrient solution was assayed weekly by the isobutyl alcohol method in which soluble P is determined spectrophotometrically as described by Watanabe & Olsen (1962). At 3 weeks, plants were harvested and analysed.

Experiment 3. The effect of foliar applied P on cluster root formation was investigated by dipping shoots of 2-month-old seedlings in solution containing P as KH_2PO_4 at 0, 0·125, 0·25, 0·5 M for 30 min. Seedlings were then transferred to $\frac{1}{2}$ l jars containing modified $\frac{1}{4}$-strength Hoagland's solution lacking P. There were 2 jars (6 plants) for each foliar treatment and they were maintained in the greenhouse as with previous experiments. At 3 weeks, foliar P was reapplied in the same manner as the initial treatment and after a further 2 weeks plants were harvested.

Experiment 4. In the previous experiments, seedlings had not been inoculated with *Frankia* but were supplied with nitrate. In the following study, the effect of nitrogen supply (either as symbiotically fixed dinitrogen or as added nitrate) was investigated. Seedlings were transferred to 1 l jars containing $\frac{1}{4}$-strength Hoagland's with 1 mg l^{-1} P. All the seedlings were then inoculated with *Frankia* isolate $HFPMg^{+}I5$ (Silvester *et al.*, 1988). Within 3 weeks after inoculation, all the seedlings were nodulated. To compare cluster root formation between nitrogen-fixing plants and nitrate-supplied plants, at varying P levels, the jars were prepared as follows: 9 jars (27 plants) were supplied with nitrate, with the aim of inhibiting nitrogen fixation and, of these, 3 jars (9 plants) were supplied with no P, 3 with 0·5 mg l^{-1} and 3 with 1 mg l^{-1} P. The remaining 9 jars with nodulated, nitrogen-fixing plants were not supplied with nitrate but were treated with the different initial P levels. The experiment was then extended for a further 3 weeks with the nutrient solution replaced every week to prevent deficiency in other nutrients.

Analysis of plant material

At harvest, plants from each experiment were separated into shoot, cluster roots and non-cluster root fractions. Cluster roots were counted and all components dried at 70 °C for at least 48 h and weighed. The dried plant tissues were then digested in perchloric acid and P content determined spectrophotometrically as described by Allen (1940).

Nitrogenase assay

For expt 4, nitrogenase activity of the plants was assayed by the acetylene reduction method (Burris, 1974) before analysing plant tissue as for all other treatments. Whole plants were individually incubated in 10% acetylene (v/v generated from calcium carbide) for 60 min at 24 °C, in 240 ml Mason jars. At the end of the assay, 0·1 ml of gas was

withdrawn for ethylene analysis on a Carle 9500 gas chromatograph equipped with a Porapak T column and flame ionization detector.

RESULTS

Morphology of cluster roots

The root system of *M. cerifera* consists of a tap root, from which arise numerous lateral roots. In the absence of available P, there are clusters of rootlets formed at regular intervals along the lateral roots (Fig. 1*a*). These cluster roots are consistent with those described for the Proteaceae (Lamont, 1972*a*) and the legumes, *Lupinus* (Gardner, Parberry & Barber, 1982) and *Viminaria* (Walker & Pate, 1986), with variation in dimensions. On *M. cerifera*, cluster roots emerge sequentially on main lateral roots with the older clusters nearer to the lateral root attachment, varying from 1–4 clusters per lateral root, depending on the length of the laterals (Fig. 1*b*). Once the first clusters appear, there is continual formation along the root system; young clusters are white and turn grey to brown with maturity over a period of 6–10 days. Clusters are not branched and range from 2 to 10 mm in length. Rootlets in clusters are initiated opposite protoxylem poles as are lateral roots in non-cluster root regions. However, cluster rootlets are closely contiguous within the axis of a cluster while lateral roots arise randomly and at a reduced frequency along the root. Hence, in contrast to the average of 1 lateral root per 8 mm of non-cluster root length, there is an average of 6 rootlets per mm of cluster root length, determinate in length, averaging 1–3 mm, and frequently bearing numerous root hairs.

Effect of P nutrition on cluster root formation

In the mineral nutrient deficiency experiment (expt 1), cluster roots were only present on plants in the solution lacking P (Fig. 1*a*). The shoots of these plants were stunted in growth as compared to the other treatments but did not show any deficiency symptoms. While morphological symptoms typical of the other nutrient deficiencies were evident in the other treatments, cluster roots were not formed in any of them.

Over the duration of expt 2, levels of P gradually decreased to zero in jars initially supplied with 0·25 or 0·5 mg l^{-1} P, while at 3 weeks P was still present in jars supplied with 1 mg l^{-1} P (Table 1). The number of cluster roots per plant decreased significantly ($P < 0{\cdot}05$) with increasing P and cluster root formation was totally suppressed in solutions supplied

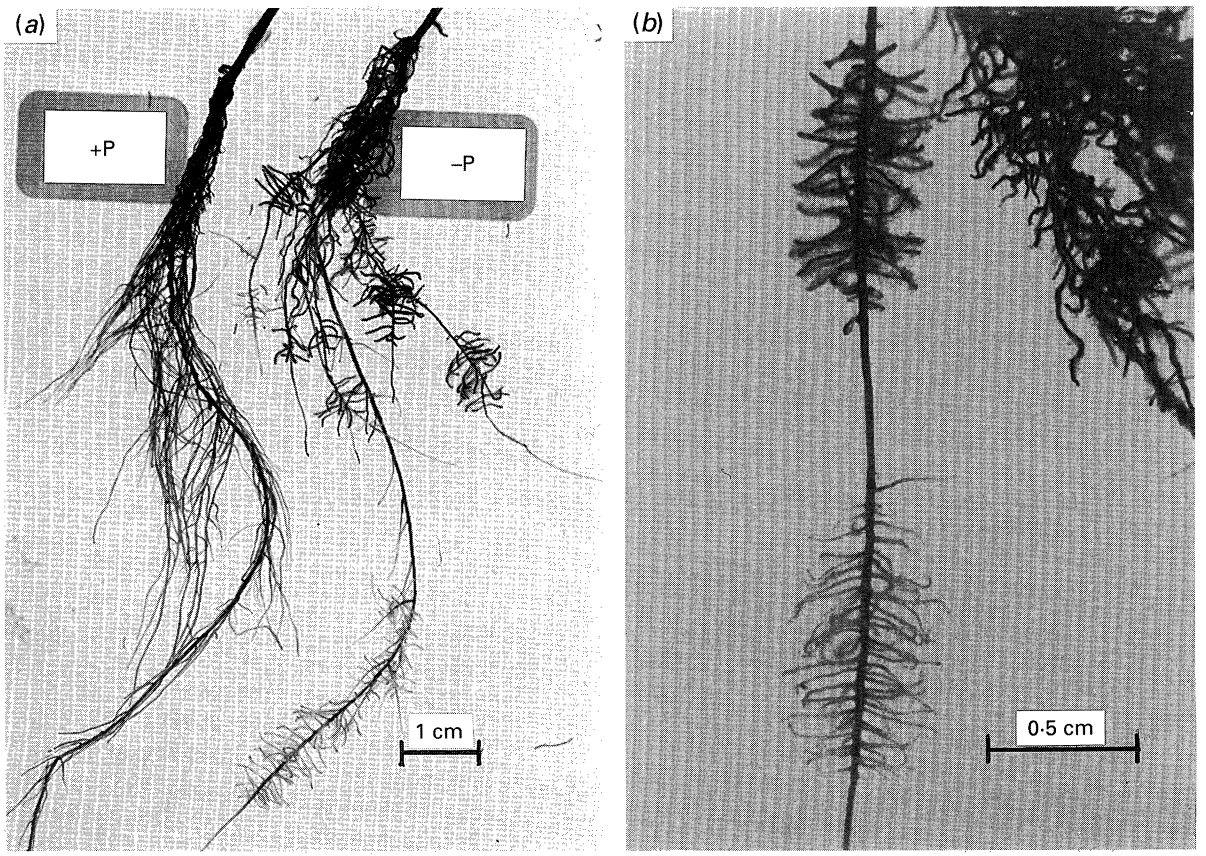

Figure 1. *Myrica cerifera* seedlings grown in $\frac{1}{4}$-strength Hoagland's solution with and without phosphorus (P). (*a*) After 3 weeks, cluster roots developed only in solutions lacking P. (*b*) Clusters form sequentially along elongating lateral roots; (root tip elongation is toward the bottom of the picture). Younger clusters are white and turn grey to brown with maturity.

Table 1. *Changes with time of phosphorus (P) concentrations in nutrient solutions maintaining* Myrica cerifera *seedlings*

	Concentration of P (mg l^{-1})			
Weeks	0	0·25	0·5	1·0
0	0	0·25 ± 0·00*	0·50 ± 0·01	1·00 ± 0·01
1	0	0·09 ± 0·01	0·22 ± 0·02	0·65 ± 0·02
2	0	0·04 ± 0·00	0·08 ± 0·01	0·41 ± 0·04
3	0	0	0	0·19 ± 0·03

* Values are means of three replicates ± SE.

with $\geqslant 1$ mg l^{-1} P (Table 2). The first clusters formed after 10 days in solution lacking P, 14 days in 0·25 ml^{-1} supplied P and 16 days in 0·5 mg l^{-1} P.

With increasing P supply, there was no significant variation in total root dry weight but there was a significant ($P < 0·05$) decrease in cluster root dry weight, with suppression of cluster roots in plants supplied 1 mg l^{-1} P. The P concentration in the roots of P-stressed plants was significantly ($P < 0·05$) lower than the P concentration of roots receiving higher P supply. Shoot dry weight increased significantly ($P < 0·05$) when P supply was increased from 0 to 0·25 mg l^{-1} P but there was no further significant increase with higher levels of available P. The P concentration in shoots, however, did increase significantly ($P < 0·05$) with increasing P supply.

In the treatments with foliar-applied P (expt 3), cluster root numbers decreased significantly ($P < 0·05$) with increasing P supply and were totally suppressed in plants supplied with 0·5 M P (Table 3). The first clusters developed after 14 days on plants supplied with no P and after 16 days in plants supplied with 0·125 and 0·25 M P. Cluster root dry weight decreased significantly with increasing P supply but there was no significant difference in total root dry weight, root P concentration and shoot dry weight.

Nitrogen, provided either as dinitrogen from the atmosphere or as nitrate to nodulated plants (expt 4), did not have any significant effect on cluster root formation (Fig. 2). Phosphorus levels, however, did have a significant ($P < 0·05$) effect on cluster root

Table 2. *Effect of varying phosphorus (P) supply in nutrient solution on cluster root formation and plant growth of* Myrica cerifera *seedlings after 3 weeks in water culture*

Concentration of P (mg l^{-1})...	0	0·25	0·5	1·0
Cluster root frequency/plant	8·63 a	7·38 a	4·38 b	0 c
Cluster root d. wt (g)	0·002 a	0·003 a	0·001 b	0 c
Shoot d. wt (g)	0·021 a	0·038 b	0·045 b	0·038 b
Total root d. wt (g)	0·008	0·010	0·011	0·010 n.s.†
% Cluster root d. wt/ total root d. wt ratio	23·00 a	30·00 a	9·09 b	0 c
Shoot P concentration (mg g^{-1} d. wt)	0·43 a	0·92 b	2·11 b	2·65 c
Root P concentration (mg g^{-1} d. wt)	0·38 a	1·70 b	1·36 b	2·60 c

* Values in each horizontal row followed by different letters indicate statistically significant differences at $P < 0·05$ (Duncan's Multiple Range Test).

† n.s. Values not significantly different at $P < 0·05$.

Each value is a mean of nine replicates.

Table 3. *Effect of foliar applied phosphorus (P) on cluster root formation and plant growth in* Myrica cerifera *after 5 weeks in water culture*

Concentration of P (M)...	0	0·125	0·25	0·5
Cluster root frequency/plant	21·6 a	14·8 a b	7·8 b c	0 c
Cluster root d. wt (g)	0·009 a	0·005 a	0·0046 a b	0 b
Shoot d. wt (g)	0·11	0·07	0·18	0·10 n.s.†
Total root d. wt (g)	0·04	0·03	0·06	0·03 n.s.†
% Cluster root d. wt/ total root d. wt ratio	22·5 a	16·67 a	7·67 b	0 c
Root P concentration (mg g^{-1} d. wt)	1·23	1·00	1·20	1·43 n.s.†

* Values in each horizontal row followed by different letters indicate statistically significant differences at $P < 0·05$ (Duncan's Multiple Range Test).

† n.s., Values not significantly different at $P < 0·05$.

Each value is a mean of six replicates.

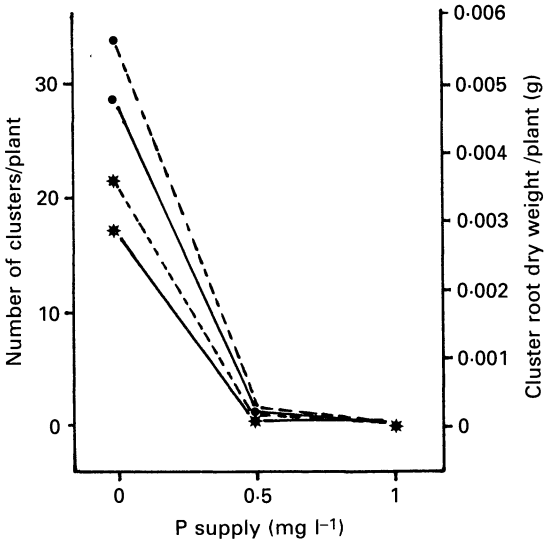

Figure 2. Effect of two nitrogen treatments, nitrate (*) and atmospheric dinitrogen (●) and varying levels of phosphorus (P) supply on nodulated *Myrica cerifera* after 3 weeks in water culture. Cluster root numbers (——) and cluster dry weight (– – –) per plant are significantly different between P levels but not between nitrogen treatments at $P < 0{\cdot}05$. Results are averages of six replicates.

formation; increasing P supply decreased cluster root numbers and dry weight, with total suppression in plants supplied with 1 mg l^{-1} P. Nitrogenase activity per g nodule was not significantly different between the two nitrogen treatments but nodule weight was significantly ($P < 0{\cdot}05$) higher in the non-nitrate supplied plants (Fig. 3*a*); consequently, nitrogenase activity was significantly higher in these plants (Fig. 3*b*).

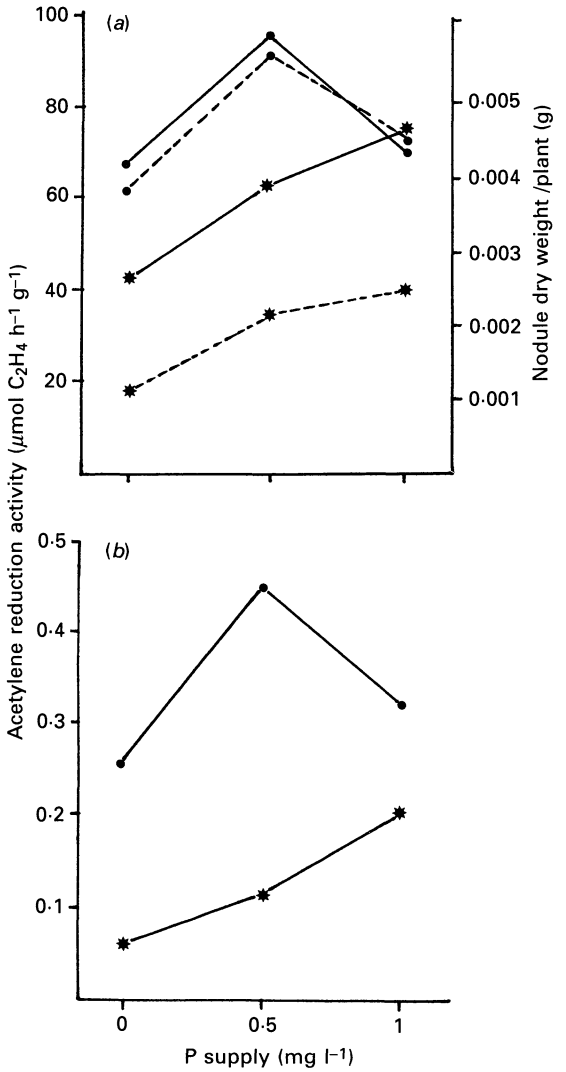

Figure 3. Effect of two nitrogen treatments, nitrate (*) and atmospheric dinitrogen (●), and varying levels of phosphorus (P) supply on nodulated *Myrica cerifera* after 3 weeks in water culture. (*a*) Nodule weight (– – –) is significantly higher in non-nitrate treatments ($P < 0{\cdot}05$) but nitrogenase activity per g nodule (—) is not significantly different ($P < 0{\cdot}05$) between nitrogen treatments. (*b*) Nitrogenase activity (acetylene reduction per g nodule dry weight) (—) is significantly ($P < 0{\cdot}05$) higher in non-nitrate treatments. Results are averages of six replicates.

DISCUSSION

Cluster root occurrence and the influence of phosphorus on its formation has been demonstrated in plant groups other than the Proteaceae (Lamont, 1982; Gardner *et al.*, 1982; Walker & Pate, 1986), but this is the first report of its occurrence on a species of the Myricaceae. These studies also clearly demonstrate that cluster root formation on *M. cerifera* is primarily influenced by phosphorus deficiency. The lower the P level of the nutrient solution the earlier the onset of cluster root formation and an initial supply of 1 mg l^{-1} P is sufficient to suppress their development in water culture for at least 3 weeks. As the total root weight did not vary significantly between P levels, but cluster root numbers and weight did, it appears that it is the proportion of cluster roots versus non-cluster roots of a root system that varies with phosphorus supply. It has been suggested that cluster root formation occurs in response to a generally low P status within the plant which may then affect the concentration of rootlets in a given portion of the root system (Jeffrey, 1967; Lamont, 1982). The studies reported here using foliar-applied P support this view that the contribution of cluster roots and non-cluster roots to the root system is determined by the internal plant P content rather than P in the surrounding ambient solution.

Although the formation of cluster roots is induced by P deficiency, the regulating mechanism involved in this is still not known. A number of factors may be involved in the phenomenon of P availability based on the physical nature and chemical reactions of the plant–soil interactions. Just as root hair length can significantly affect plant P nutrition (Foehse & Jungk, 1983), so could clustering of rootlets. Malajczuk & Bowen (1974) suggested that enhanced P

uptake by microbes in the rhizosphere limited P supply to the roots and induced rootlet clustering in the Proteaceae. While the role of microbes needs to be more fully studied, the effect of low P on cluster root formation is now clearly apparent.

The function of cluster roots in relation to P nutrition is still not understood. Cluster roots may not be involved directly in the uptake of P but in the modification of the rhizosphere to enhance P mobilisation towards the plant. Release of exudates from the root is one such adaptive mechanism by which P-stressed plants can alter their environment and subsequently affect nutrient availability in the rhizosphere. Gardner, Barber & Parberry (1983) have suggested that clustered root zones in lupins are the sites of increased citrate exudation which would correspond with increased acidification of the rhizosphere and P mobilization by the plant. Sufficient plant P content however suppresses this root clustering effect.

As the Proteaceae and lupins appear to be nonmycorrhizal, it has been suggested that cluster roots are an alternative to mycorrhizas in enhancing phosphorus uptake (Trinick, 1977; Gardner, Parberry & Barber, 1981). The mycorrhizal status of the Myricaceae is still far from clear, although both vesicular–arbuscular mycorrhizas and ectomycorrhizas have been reported on *M. cerifera* (Rose, 1980). Inoculation of seedling with various mycorrhizal fungi resulted in no infection in studies at the Harvard Forest, but this may be because a particularly beneficial symbiont for *M. cerifera* has yet to be identified. Although the suppression of cluster roots at high P levels is similar to the suppression of mycorrhizas by raised P levels, the significant increase in cluster root formation in water culture in minus P conditions is unlike the initiation of mycorrhizal symbiosis which is most effective at low P levels. This comparison again suggests that cluster roots may not be directly involved in P uptake.

Unlike the Proteaceae, cluster-rooted plants in the legumes and actinorhizal plants are associated with symbiotic nitrogen-fixing micro-organisms (*Rhizobium* and *Frankia*, respectively). Addition of nitrate to nodulated *M. cerifera* plants resulted in a reduction of further nodule development and nitrogen-fixation, thus supporting previous reports of nitrate inhibition of existing nodules on actinorhizal plants (Kohls & Baker, 1989). However, provision of nitrogen as nitrate or as dinitrogen via nitrogenase did not have any effect, directly or in relation to varied phosphorus levels, on cluster root formation in *M. cerifera*. It thus does not appear that the capacity of actinorhizal plants to fix nitrogen by symbiotic association with a soil micro-organism is of any significance in cluster root formation.

While abundant root hair development and extensive mycorrhizal mycelium distribution in the rhizosphere are accepted as the major means for improved plant P uptake in soils of limited nutrient supply, the significance of rootlet clustering in relation to phosphorus nutrition and the mechanism involved in its induction in *M. cerifera* and its related members in actinorhizal plants warrant further investigation.

ACKNOWLEDGEMENTS

The authors acknowledge support for this research from the USDA Competitive Grants Programme (Research Grant 88-33520-4071) and by a grant from the A. W. Mellon Foundation of New York. We are grateful for technical assistance from Ralph Lundquist.

REFERENCES

ALLEN, R. J. L. (1940). The estimation of phosphorus. *Biochemistry Journal* **34**, 858–865.

BURRIS, R. H. (1974). Methodology. In *The Biology of Nitrogen-fixation* (Ed. by A. Quispel), pp. 9–33. North-Holland Publishing Co., Amsterdam, The Netherlands.

FOEHSE, D. & JUNGK, A. (1983). Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant and Soil* **74**, 359–368.

GARDNER, W. K., PARBERRY, D. G. & BARBER, D. A. (1981). Proteoid root morphology and function in *Lupinus albus*. *Plant and Soil* **60**, 143–147.

GARDNER, W. K., PARBERRY, D. G. & BARBER, D. A. (1982). The acquisition of phosphorus by *Lupinus albus* L. II. The effect of varying phosphorus supply and soil type on some characteristics of the soil/root interface. *Plant and Soil* **68**, 33–41.

GARDNER, W. K., BARBER, D. A. & PARBERRY, D. G. (1983). The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. *Plant and Soil* **70**, 107–124.

HOAGLAND, D. R. & ARNON. D. I. (1950). The water culture method for growing plants without soil. California Agricultural Experimental Station, Circular **347.**

JEFFREY, D. W. (1967). Phosphate nutrition of Australian heath plants. I. The importance of proteoid roots in *Banksia* (Proteaceae). *Australian Journal of Botany* **15**, 403–411.

KOHLS, S. J. & BAKER, D. D. (1989). Effects of substrate nitrate concentration on symbiotic nodule formation in actinorhizal plants. *Plant and Soil* **118**, 171–179.

LAMONT, B. (1972*a*). The morphology and anatomy of proteoid roots in the genus *Hakea*. *Australian Journal of Botany* **20**, 155–174.

LAMONT, B. (1972*b*). The effect of soil nutrients on the production of proteoid roots by *Hakea* species. *Australian Journal of Botany* **20**, 27–40.

LAMONT, B. (1972*c*). 'Proteoid roots' in the legume *Viminaria juncea*. *Search* **3**, 90–91.

LAMONT, B. (1982). Mechanisms for enhancing nutrient uptake in plants, with particular reference to Mediterranean South Africa and Western Australia. *Botanical Review* **48**, 597–698.

LAMONT, B. (1983). Proteoid roots in the South African Proteaceae. *Journal of South African Botany* **49**, 103–12.

MACHLIS, L. & TORREY, J. G. (1956). *Plants in Action: A Laboratory Manual of Plant Physiology*, W. H. Freeman & Co., San Francisco, USA.

MALAJCZUK, N. & BOWEN, G. D. (1974). Proteoid roots are microbially induced. *Nature* **251**, 316–317.

PATHMARANEE, N. (1974). *Observations on proteoid roots*. M.Sc. Thesis, University of Sydney, New South Wales.

PURNELL, H. M. (1960). Studies of the family Proteaceae. I. Anatomy and morphology of the roots of some Victorian species. *Australian Journal of Botany* **8**, 38–50.

REDELL, P. (1986). *Soil and plant factors affecting nodulation and nitrogen-fixation in Casuarinaceae*–Frankia *symbiosis*. Ph.D. Thesis, University of Western Australia, Perth.

ROSE, S. L. (1980). Mycorrhizal associations of some actinomycete nodulated nitrogen-fixing plants. *Canadian Journal of Botany* **58**, 1449–1454.

SILVESTER, W. B., WHITBECK, J., SILVESTER, J. K. & TORREY, J. G. (1988). ,Growth, nodule morphology and nitrogenase activity of *Myrica gale* with roots grown at various oxygen levels. *Canadian Journal of Botany* **66**, 1762–1771.

TRINICK, M. J. (1977). Vesicular–arbuscular infection and soil phosphorus utilization in *Lupinus* spp. *New Phytologist* **78**, 297–304.

WALKER, B. A. & PATE, J. S. (1986). Morphological variation between seedling progenies of *Viminaria juncea* (Schrad & Wendl) Hoffmans (Fabaceae) and its physiological significance. *Australian Journal of Plant Physiology* **13**, 305–319.

WATANABE, F. S. & OLSEN, S. R. (1962). Colorimetric determination of phosphorus in water extracts of soil. *Soil Science* **93**, 183–188.