Cluster root formation by *Gymnostoma papuanum* (Casuarinaceae) in relation to aeration and mineral nutrient availability in water culture

Suzanne Racette, Isabelle Louis, and John G. Torrey

Harvard Forest, Harvard University, Petersham, MA 01366, U.S.A.

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The term cluster root is used to refer to a dense cluster of determinate lateral roots (rootlets), in preference to the terms proteoid root and proteoid-like root used by other authors. Cluster roots are often formed by the actinorhizal plant *Gymnostoma papuanum*. In water culture, cluster root formation by *G. papuanum* was influenced by aeration, phosphorus level, and nitrogen source. Aeration was a critical factor, with nonaerated rooted cuttings having far fewer cluster roots than aerated ones. Phosphorus deficiency was the single nutrient deficiency that led to increased cluster root formation. Seedlings, grown under conditions of either low (0.8 mg L\(^{-1}\)) or no phosphorus, responded by devoting a greater portion of root growth to the production of cluster roots, with no overall reduction in root growth for 6 weeks. The response to varying phosphorus level was modified by providing nitrogen in different forms. Supplying nitrogen as ammonium resulted in low levels of cluster root formation. Supplying nitrate to nodulated seedlings led to an increase in cluster root formation in comparison with plants that depended solely upon dinitrogen fixation by *Frankia*. Greatest cluster root formation occurred on plants grown in aerated water cultures supplied with nitrate and with little or no phosphorus.

**Key words:** Gymnostoma papuanum, cluster roots, proteoid roots, phosphorus deficiency.


Les auteurs utilisent le terme racines groupées (« cluster root ») préférentiellement au terme racines protéoides ou de forme protéoides, utilisés par d’autres auteurs pour désigner des amas denses de racines latérales déterminées (radicelles). Les racines groupées sont fréquentes chez la plante actinorhizienne Gymnostoma papuanum. La formation de racines regroupées chez le *G. papuanum* cultivé dans l’eau est influencée par la quantité de phosphore et la source d’azote. L’aération est un facteur critique; avec d’aérateurs, les plantules forment moins de racines groupées que les plantules aérées. La déficience en phosphore est la seule déficience nutritive qui conduit à une augmentation des racines groupées. Les plantules cultivées sous des conditions de faible teneur (0.8 mg L\(^{-1}\)) ou d’absence en phosphore, réagissent en développant une forte proportion de racines groupées, sans qu’il y ait réduction globale dans la croissance racinaire sur une période de 6 semaines. La réaction obtenue en variant la teneur en phosphore est modifiée par l’addition d’azote sous des formes différentes. L’azote apporté sous forme d’ammonium réduit la quantité de racines groupées. L’apport de nitrate sur des plants nodule conduisait à une augmentation des racines groupées comparativement aux plantes qui dépendent seulement de la fixation de l’azote diatomique fixé par le *Frankia*. La plus forte formation de racines groupées apparait sur les plantes cultivées dans l’eau aérée additionnée de nitrate et avec de petites quantités ou pas du tout de phosphore.

**Mots clés:** Gymnostoma papuanum, racines groupées, racines protéoides, déficience en phosphore.

**Introduction**

In describing the morphology of roots of some species from the family Proteaceae, Purnell (1960) defined a “proteoid root” as a “cluster of rootlets, of limited growth, which forms on a lateral root.” Proteoid roots are commonly found on a majority of species in the Proteaceae (Lamont 1983), and factors that influence their formation have been extensively examined (Lamont 1972a, 1972b, 1973, 1976; Lamont and McComb 1974; Malajczuk and Bowen 1974; Lamont et al. 1984). In 1982, Lamont introduced the use of the general term “root clusters,” which he defined as bunches of hairy rootlets produced on occasion by certain species and including proteoid and proteoid-like roots. We prefer to substitute the term cluster root for the more specific term proteoid root.

Cluster roots have been described for a number of species from families other than the Proteaceae. These plants include the legumes *Lupinus consenitini* (Trinick 1977), *Lupinus albus* (Gardner et al. 1981), and *Viminaria juncea* (Lamont 1972c), and the actinorhizal plants *Casuarina* spp. (Redsell 1986) and *Myrica cerifera* (Louis et al. 1990). Phosphorus (P) in the root environment is an important factor in the production of cluster roots in several of these non-Proteaceae species. Trinick (1977) observed a decrease in their formation on *L. consenitini* with increasing P level, and Walker and Pate (1986) reported the same for *V. juncea*. Similarly, cluster root formation on *L. albus* is influenced predominantly by the P status of the plant (Gardner et al. 1982a). For *M. cerifera*, levels of P as low as 1 mg L\(^{-1}\) suppress the formation of cluster roots in hydroponics (Louis et al. 1990). *Gymnostoma papuanum* (S. Moore) L. Johnson, an actinorhizal tree species in the family Casuarinaceae, falls within the category of plants that bear cluster roots. Cluster roots occur on hydroponically, aeroponically, and pot-grown plants in the greenhouse at Harvard Forest in Petersham. This study shows the effects of aeration, P supply, and nitrogen (N) source on cluster root formation on hydroponically grown *G. papuanum.*

**Materials and methods**

The following experiments on cluster root formation by *G. papuanum* were carried out in water culture using modified Hoagland’s nutrient solution as specified for each experiment. At the time that experiment I was initiated, seed of *G. papuanum* was not available, therefore rooted cuttings were utilized. Seeds were later obtained and seedlings were then used for experiments 2–4. For all experiments,
the harvest of each plant included counting the number of cluster roots and nodules (when present) and then separating shoot, nonclustered roots, cluster roots, and nodules, drying them for 2 days at 70°C, and then determining dry weights. Data were analysed with the assistance of the statistical software ABStat® (Anderson-Bell, Canon Bell, Colorado City, CO).

**Experiment 1**

Experiment 1 examined the effects of aeration and varying P levels on cluster root formation. Adventitious roots were induced on cuttings of *G. papuuanum* according to procedures of Lundquist and Torrey (1984). Rooted cuttings were transferred to water culture jars (three per jar) and placed in a temperate-environment greenhouse. Each jar contained 1 L of quarter-strength Hoagland's nutrient solution, − N, pH 7.0 (Hoagland and Arnon 1950). All plants were inoculated with *Frankia* isolate HFPGpIl (Racette and Torrey 1989). When nodules had been initiated on over 50% of the plants (4 weeks), the nutrient solution was replaced with treatment solutions (six jars per treatment), and half of the jars of each treatment were aerated using an aquarium pump and plastic tubing. Treatment solutions consisted of quarter-strength Hoagland's, − N, pH 7.0, with various P levels. P levels were selected based on the amount of P normally in quarter-strength Hoagland's (8 mg L⁻¹). The four P levels used were 16, 8, 0.8, and 0 mg L⁻¹. At 16 mg L⁻¹ P, additional P was supplied as NaH₂PO₄ at 0 and 0.8 mg L⁻¹ P, KCl replaced the KH₂PO₄, which was eliminated. Nutrient solutions were changed every 2 weeks. Plants were harvested 15 weeks after treatments began.

**Experiment 2**

Experiment 2 tested the effect of using different N sources and P levels on cluster root formation. Seedlings of *G. papuuanum* were established from seed collected at the Waiaka Arboretum in Hawaii using procedures described by Racette and Torrey (1989). Seedlings were placed in 0.5-L water culture jars, three plants per jar. All jars were placed in a growth chamber on a 16 h light : 8 h dark cycle at 33:28°C (light:dark), and all were aerated. For this and following experiments, the formulations of Hoagland's nutrient solutions used were based on Machlis and Torrey (1956), made up at quarter-strength. Treatments consisted of varying P levels and N sources. P levels used were the same as in experiment 1. For each P level, three jars received quarter-strength Hoagland's nutrient solution with NO₃, three jars received an NH₄-containing solution, and plants in three jars were inoculated with *Frankia* isolate UFGC15 (kindly provided by H. Berg) and received a − N nutrient solution. For the NH₄ treatment, (NH₄)₂SO₄ was substituted for NO₃ at a level that supplied a N molarity equivalent to the NO₃-containing solution (3.75 mM). Solutions were replaced once, after 4 weeks, and plants were harvested after 6 weeks. Nine plants were started for each treatment, but only six from each treatment were harvested because of seedling death during the experiment.

**Experiment 3**

Experiment 3 examined cluster root formation of nodulated seedlings growing at different P levels, either with or without added nitrate. Seedlings were set up in 0.5-L water culture jars containing quarter-strength Hoagland's nutrient solution, − N, 0.8 mg L⁻¹ P, pH 7.0, and inoculated with *Frankia* isolate UFGC15. All jars were placed in the greenhouse and aerated. Solutions were changed after 4 weeks and after 6 weeks were replaced with treatment solutions, three jars per treatment. Nodulation was confirmed on all plants before treatments were started. Formation of nodule roots had begun on some plants. Treatments consisted of quarter-strength Hoagland's solution modified to contain three P levels (8, 0.8, and 0 mg L⁻¹) in combination with two N treatments (± NO₃). Solutions were changed after 2 and 4 weeks of treatment, and plants were harvested 6 weeks after treatments began (eight plants per treatment). An acetylene reduction assay (Burris 1974) was used to determine the degree of suppression of nitrogenase activity due to the addition of nitrate to the nutrient solution. Immediately prior to harvest, individual plants were incubated in 10% acetylene for approximately 1 h, and the amount of ethylene produced was measured on a Carle 9500 flame ionization gas chromatograph.

**Experiment 4**

Experiment 4 tested the effects of a range of nutrient deficiencies on cluster root formation. Seedlings were placed in 0.5-L jars containing the following modified quarter-strength Hoagland's solutions: − S, − Mg, − K, − Fe, − Ca, − P, − N, and − trace elements (formulations based on Machlis and Torrey 1956). Hoagland's complete nutrient solution at quarter-strength was run as a control, and all solutions were adjusted to pH 7.0 prior to use. Nine seedlings (three jars) were set up for each treatment. All jars were placed in a growth chamber and aerated (as for experiment 2). Solutions were changed after 2 and 4 weeks, and plants were harvested after 6 weeks.

**Results**

*Gymnostoma papuuanum* spontaneously produced cluster roots after a period of time when grown hydroponically in quarter-strength Hoagland's nutrient solution. Clusters averaged 3.3 ± 1.4 mm in length, with 12.4 ± 1.7 rootlets per mm, and individual rootlets were generally 3 mm or less in length (Fig. 1). As the following results indicate, cluster root formation can be increased or decreased by manipulation of culture conditions, specifically aeration and nutrient supply.

**Experiment 1**

In experiment 1, the increase in cluster root formation by plants grown with aerated compared with plants grown without aeration was highly significant (p < 0.01). The amount of cluster root formation increased on a per plant basis, and a
greater number of plants formed cluster roots with aeration (Table 1). Increased cluster root formation was not due to an increase in plant growth, as there was no significant difference in root or shoot dry weights as a result of either aeration or P level (Table 1). The graphs in Fig. 2 suggest that in aerated cultures, cluster root formation increased as P level decreased, although there was no statistically significant difference in cluster root number, in cluster root dry weight, or in the percentage of the total root weight made up of cluster roots due to the change in P level. Lack of statistical significance may be attributable to the high within-treatment variation that occurred despite the use of clonal plants.

**Experiment 2**

In experiment 2, both P level and N source had highly significant effects (p < 0.01) on the formation of cluster roots on seedlings (Fig. 3). In general, decreasing P level led to increased cluster root development regardless of the N source, but this effect was most pronounced when NO₃⁻ was supplied. Although plants grew best when NH₄⁺ was the N source (Table 2), cluster root formation was eliminated at 8 and 16 mg L⁻¹ P and much reduced at the lower P levels compared with NO₃⁻-fed plants. For inoculated plants, development of cluster roots did not show as much variation with changing P level as it did for plants given fertilizer N. The inoculated plants did not nodulate until 3 weeks into the experiment, and as a result, nodulated plants were significantly smaller at the end of the experiment than in either NO₃⁻- or NH₄⁺-fed plants (Table 2). Whether this affected cluster root development could not be determined, but there did not appear to be any significant lag time in the onset of cluster root development between N treatments. In relation to root growth, although decreasing P level led to increased cluster root formation, there was no significant change in total root weight due to the change in P supply. This result indicates that the decrease in P resulted in a change in the type of root growth occurring, not in the total amount of root growth.

**Experiment 3**

Experiment 3 was designed to determine whether the reduction in cluster root formation on inoculated versus NO₃⁻-fed plants in experiment 2 would remain if all plants were first inoculated. The plan was to eliminate from the experimental period the N-free time that occurred during the nodulation process and thereby equalize growth among treatments. Results in Table 3 suggest that this effect was accomplished, as there was no significant difference in root or shoot dry weights owing to N treatments. The addition of NO₃⁻ to nodulated seedlings did result in a decrease in final nodule number and nodule dry weight per plant, as well as a significant decrease in nitrogenase activity, both per gram of nodule dry weight and per plant, but did not lead to an increase in plant growth. Although all plants grew equally well, the graphs in Fig. 4 clearly indicate that plants supplied with NO₃⁻ formed significantly more cluster roots in comparison with those which depended on N₂ fixed by Frankia. The relation between P level and cluster root formation is not as clear as in experiment 2, but there was still a significant increase in cluster root dry weight and the percentage of root expressed as cluster roots at the 0 P level (p < 0.05). The lack of more significant variation in relation to P level may relate to the 6-week pretreatment period in which all plants received 0.8 mg L⁻¹ P.

**Experiment 4**

In this experiment, cluster root formation was significantly greater on plants grown in a solution lacking P, compared with all other treatments (Duncan’s multiple range test, p < 0.01), as measured by cluster root dry weight and by the percentage of the total root weight made up of cluster roots (Fig. 5). Cluster roots that formed in −P were generally larger than clusters in other treatments, with each cluster having more roots. The number of cluster roots varied tremendously among plants for some treatments, including the control where with a mean of 13.7 clusters per plant, there was a range of 0–76 clusters per plant, resulting in a standard deviation of 24.2. While the highest mean number of cluster roots per plant occurred with −P (26.3), the large within-treatment variation resulted in no significant difference in cluster root numbers among the control, −P, −Ca, and −Mg treatments.

**Discussion**

The formation of cluster roots by *G. papuanum* grown in water culture is highly dependent on growing conditions and nutrient supply. Three factors, P supply, aeration, and N source, markedly influence the amount of cluster root formation that occurs. Each of these factors will be discussed in turn.
While the results of the nutrient-deficiency experiment do not rule out an influence of other nutrients, only when P was the missing nutrient was there a significant increase in cluster root formation. *Gymnostoma papuanum* responds to a deficiency of P by devoting a greater portion of root growth to the formation of cluster roots. This observation fits the hypothesis of Jeffrey (1967) that proteoid roots form as a response to the low-P status of plants. Increase in cluster root formation due to decreasing P availability has been clearly shown for several
species other than *G. papuanum*, but the response is somewhat variable. A second actinorhizal plant for which the response to P has been studied is *M. cerifera* (Louis et al. 1990). *Myrica cerifera* forms cluster roots at very low P levels, with as little as 1 mg L\(^{-1}\) P in the nutrient solution of hydroponically grown plants totally suppressing cluster root formation. In contrast, *G. papuanum* forms cluster roots at 16 mg L\(^{-1}\) P, more closely resembling cluster root formation of the legumes *V. juncea* (Walker and Pate 1986) and *L. albus* (Gardner et al. 1982b), which show decreasing cluster root formation with increasing P fertilization but still form some cluster roots when grown in water culture. While *G. papuanum* is relatively slow growing, significant growth did occur during all experiments. These results suggest that both seedlings and rooted cuttings of *G. papuanum* require only low amounts of P for growth, and the plants are able to provide the necessary amount of P from

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internal reserves for extended periods of time when external supplies are limited. Thus, abundant cluster root formation may serve as an indicator of low external P supply when growth-related P deficiency symptoms are not evident in *G. papuanum*.

The effects of aeration on cluster root formation were statistically highly significant, with an increase in both the number of plants that formed cluster roots and the amount of cluster root formation per plant when cultures were aerated. The dramatic increase that occurred does not appear to be directly related to growth rate, as nonaerated plants were on average the same size as aerated ones. This fact suggests that there may be an additional requirement of the root for air (oxygen) to form cluster roots beyond that required for normal growth and maintenance. The formation of cluster roots involves a large amount of meristematic activity and cell growth, and presumably, therefore, a high respiration rate within localized short segments of root. It is possible that the need for aeration thus relates to a higher demand for oxygen at the time and sites of cluster root development. These results correlate well with the observations of Lamont (1976) who showed that maximum proteoid root numbers on *Hakea* occurred under conditions of moderate waterlogging, decreasing if water availability increased (oxygen decreased).

Lamont (1972a) reported that of the factors studied, N concentration appeared to have the greatest effect on determining the relative contribution of proteoid roots to the root system of *Hakea*. Later, Lamont (1973) demonstrated that proteoid root production was decreased by an increase in N status of shoots of *Hakea* plants. Since *G. papuanum* forms a symbiotic relationship with the dinitrogen-fixing bacterium *Frankia*, the relationship of N to cluster root formation is particularly interesting. In our experiments, the effect of three different sources of N was examined. The results indicate that the source of N influences the formation of cluster roots to a significant degree.

**FIG. 5.** Total root dry weight and dry weight of cluster roots formed on seedlings of *G. papuanum* grown in water-culture solutions deficient in mineral nutrients as noted. TE, trace element; +, complete nutrient solution. Numbers within bars are the percentages of total root weight consisting of cluster roots. Vertical lines represent ± SE.
Ammonium was the best source of N for growth, but its use led to the lowest level of cluster root production. If, in this case, the greater growth of ammonium-fed plants indicates a higher shoot N status, then these data agree with the results of Lamont’s work. The difference between plants receiving nitrate N and those growing solely with dinitrogen fixed by Frankia is not as clear. These plants appeared to grow equally well, but those receiving nitrate produced significantly more root clusters. Further experiments using different levels of N and determining actual shoot and root N content will be necessary to better understand the relationship between N source and cluster root formation. Furthermore, the relationship between N source and cluster root formation may not be the same for other species. A similar experiment with M. cerifera showed no difference in cluster root formation between plants that did or did not receive a nitrate-containing nutrient solution (Louis et al. 1990).

While it is clear that both P and N nutrition are involved in the regulation of cluster root formation, the mechanism whereby this developmental expression occurs is not obvious. The ultimate basis for the control of cluster root formation must lie in the effects of these nutrients on factors that regulate the initiation of new lateral root meristems, including the levels of hormones such as auxin (cf. Torrey 1986). For those plants which form them, cluster roots may actually be the normal type of root growth found in the field, where P levels are often very low, and their occurrence may be much more widespread than currently reported. Of course, this leads to the question of whether or not the formation of cluster roots at low P levels is of benefit to the plant. Work on L. albus (Gardner et al. 1981, 1982a, 1982b, 1983) does suggest a possible mechanism whereby cluster roots may allow for increased P uptake by a plant. Further work with other plants that form cluster roots, perhaps including G. papuanum, will be necessary before the question of function is resolved.

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