

## Spatial Patterns of Root Branching and Actinorhizal Nodulation in *Discaria trinervis* Seedlings

EUGENIA CHAIA<sup>1\*</sup> and ESTELA RAFFAELE<sup>2</sup>

<sup>1</sup>Departamento de Biología and <sup>2</sup>Departamento de Ecología, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Quintral 1250, 8400 San Carlos de Bariloche, Argentina, Tel. +54-2944-42-3374, Fax. +54-2944-42-2111, E-mails. echaia@crub.uncoma.edu.ar and eraffael@crub.uncoma.edu.ar

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### Abstract

The spatial patterns of root branching (topology) and actinorhizal nodulation of *Discaria trinervis* seedlings under natural and controlled conditions were analysed. The link, i.e. the root segment between either two branching points, or between a meristem and a branching point, was used as a structural unit of roots. Nodulated field plants displayed a random model of root topology and experimental plants without root symbionts a herringbone model (Fitter, 1991). The occurrence of these different root topologies would suggest that root symbionts influence root model architecture. The frequency distribution of the number of nodules per link on the main roots, for field collected plants, fitted a negative binomial model. Nodules were mainly situated near the main root's proximal end. The position (number of links counted from the main root's proximal end to the root apex) of lateral nodulated roots coincided with the nodulated area of the main root. A nodulation assay showed that the spatial pattern of root nodules was not a consequence of heterogeneous distribution of infective units of *Frankia* in the soil. The similar pattern of nodule position in field plants and experimental plants suggested that a regulation of nodulation might be also working in plants in their natural environment.

Keywords: Actinorhiza, root architecture, autoregulation, *Discaria trinervis*, *Frankia*, nodulation, spatial pattern

\*The author to whom correspondence should be sent.

## 1. Introduction

*Discaria trinervis* (Gillies ex Hook. & Arn.) Reiche is a deciduous tree or shrub native to the Southern Andes, South America. It occurs in scrubs (Tortosa, 1983), disturbed sites such as roadsides and in abandoned fields, and has the ability to survive long dry periods in infertile sites (Silvester et al., 1985). In the field, the root system of *D. trinervis* is naturally infected by endomycorrhizal fungi (Fontenla, 2000) and by the actinomycete *Frankia* (Chaia, 1997), which forms dinitrogen-fixing nodules (Chaia, 1998). The infection pathway followed by *Frankia* BCU110501 in *D. trinervis* seedlings is the intercellular penetration among epidermal and cortical root cells (Valverde and Wall, 1999a). A homeostatic balance controls the initiation and development of the actinorhizal nodules (Wall and Huss-Danell, 1997; Valverde and Wall, 1999b; Valverde et al., 2000). Experimental studies showed that nodule formation in *D. trinervis* might be controlled by a feedback mechanism which follows two different initial pathways that operate through inhibition of infection and nodule development (Valverde and Wall, 1999b); further nodule growth and activity would be regulated by the internal plant nitrogen level (Valverde et al., 2000).

It was found that the growth rate of *D. trinervis* roots decreased after the onset of dinitrogen fixation, so autoregulation phenomena would be also influencing the growth of *D. trinervis* roots (Valverde et al., 2000). Control mechanisms that regulate root development have already been described for *Arabidopsis* (Zhang and Forde, 1998; Zhang et al., 1999). A valuable approach to describe the growth of a root system may be provided by the architectural analysis described by Fitter et al. (1991) and Fitter (1996). Although the pattern of root branching (topology) is influenced by the genetic make up and overall nutrient status of the plant, and the micro-environment of the soil (Leyser and Fitter, 1998), it is possible to estimate how root form and function are related and whether selective pressures in particular environments favour particular types of roots systems (Fitter, 1991; Fitter and Stickand, 1992).

Autoregulation phenomena play a role in the distribution of nodules (Valverde et al., 2000; Valverde and Wall, 1999b; Wall and Huss-Danell, 1997). There is a clear zonation of the infectible root cells in *Alnus glutinosa* (Burggraaf et al., 1983) and *A. incana* (Wall and Huss-Danell, 1997). In *D. trinervis* seedlings, inoculated and nodulated in pouches, nodules are confined to a defined region of the tap root (Valverde et al., 2000). Preliminary observations showed that the distribution of nodules along the roots of *D. trinervis* growing under natural conditions resemble that of *D. trinervis* growing under experimental conditions (Valverde and Wall, 1999b; Valverde et al., 2000). The finding of spatial patterns of root branching and nodulation would

contribute to explore the existence of autoregulation mechanisms in plants under natural conditions.

In the present paper we tested the architectural models of root systems (Fitter, 1991) in actinorhizal *D. trinervis* seedlings, in which it is predicted that exploitation efficiency is a function of root system architecture. We also describe the spatial pattern of the actinorhizal nodulation in *D. trinervis* plants under natural and experimental conditions.

## 2. Material and Methods

### *Study area*

Plant material and soil samples were collected from the alluvial plain of the Ñireco river, Pampa de Huenuleo, San Carlos de Bariloche, Argentina (41°10'S 71°21'W, 935 m above sea level). The climate of this region is humid temperate, mesothermal, with a maximum annual mean temperature of 13°C and a minimum of 2°C, mean annual rainfall is 1200 mm, with frequent winter snowfalls (Muñoz and Garay, 1985). The soil, typical Vitrandept, has a relatively poor edaphic development (Bianchi, 1987), a nitrogen content of 0.2 mg g<sup>-1</sup> dry soil (Kjeldahl method), a phosphorus content of 4.4 µg g<sup>-1</sup> dry soil (Olsen method) and pH 7.1. The vegetation is a mixed scrub dominated by *Nothofagus antarctica*, *Discaria chacaye*, *D. trinervis*, *Berberis heterophylla* and *Fabiana imbricata*.

### *Field sampling*

In March 1995, we collected *Discaria trinervis* seeds and seedlings; seedling roots were growing to a depth of ca. 9 cm. In February 1997, fifteen random sampling points were established at the sampling site and holes were dug with a clean shovel. At each hole, three soil samples at: 0–3, 3–6 and 6–9 cm depth were collected by means of a 5 ml sterile syringe, the end of which had been cut off with a blade, and which was inserted horizontally into the soil profile. The air-dried soil samples were kept in plastic bags at 4°C for one week. Additional soil samples were collected in the same site at a depth of 15 cm.

### *Root topology of plants under experimental conditions (experiment 1)*

An experiment was conducted to verify if the root topology model found in the field plants could be affected by the symbioses with *Frankia* and with endomycorrhizal fungi.

Scarified *D. trinervis* seeds were germinated in Petri dishes and transferred to 30 pots containing 80 cm<sup>3</sup> of pure soil obtained at a depth of 15 cm. An additional set of 30 pots with tyndalized soil (open flow in autoclave, 3 days for 1 h) was used to grow the negative controls. One plant was left in each pot. The seedlings were watered with distilled water. Plants were kept in a growth chamber with 16 h photoperiod (photosynthetic photon flux density ca. 70  $\mu\text{moles m}^{-2} \text{s}^{-1}$ ) at 19–24°C temperature and 40% relative humidity for five months. The roots were cleared and stained (Phillips and Hayman, 1970) to register the presence of arbuscular mycorrhizal infection. The number of nodules, root and shoot length and parameters of root topology were measured for each plant.

#### *Nodulation capacity of soil samples (experiment 2)*

An experiment was conducted to determine whether the infective units of *Frankia* are randomly distributed in the soil, at depths where roots of the collected *D. trinervis* plants were found. We compared the spatial nodulation pattern of some plants of this assay with that of field plants.

Seeds were scarified for 3 min with concentrated H<sub>2</sub>SO<sub>4</sub> (Chaia, 1998). A pool of seeds (10±5) was sown in each of 45 sterile glass tubes (200 mm long × 26 mm in diameter) filled with a mixture of sterile sand and 5 g of the field soil sample obtained at one of the depths mentioned above. Negative controls were grown in tubes filled with a mixture of sterile sand and 5 g of steam sterilised soil (twice, in autoclave 30 min at 121°C), obtained by combining samples from the different depths and the different sampling points. The tubes were capped with sterile cotton plugs and their lower parts were protected from light. Seedlings were watered with distilled water and fertilised weekly with nitrogen-free one-fourth-strength Hoagland solution (Tortosa and Cusato, 1991). After two months, the seedlings were thinned to three similarly sized plants in each tube.

Fifteen replicates per soil depth were arranged in a complete randomised design. The growth chamber had the same conditions as in the previous experiment. This assay lasted five months. After that period, nodulation, morphological appearance, number of shoot internodes and root links, and dry weight were registered for each plant. Each nodule was considered as an infective unit from the soil (Oremus, 1980).

#### *Architectural analysis*

The roots of *D. trinervis* seedlings were described by using the parameters of root systems architecture (Fitter, 1991). The link, i.e. the root segment either

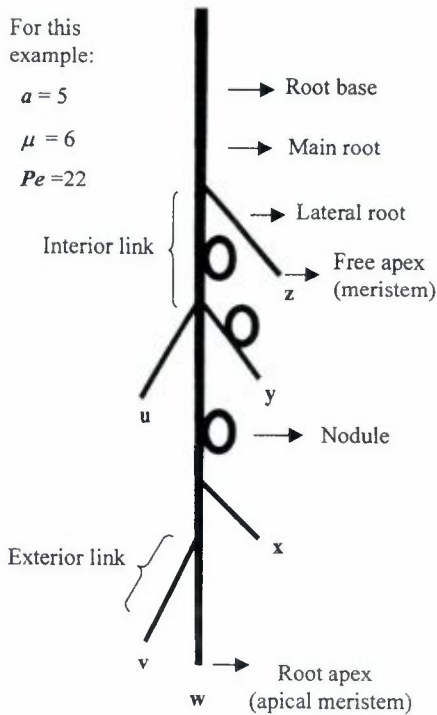


Figure 1. Diagram of a root showing the architectural root parameters described by Fitter (1991).  $a$ : altitude (number of links in the main root, i.e. the longest path from the root base to any link ending in a free apex or meristem: exterior link),  $\mu$ : magnitude (total number of exterior links, i.e. number of links determined by a branch at one end and a meristem at the other),  $Pe$ : total exterior pathlength (sum of the pathlengths for all exterior links, i.e. adding links from:  $z$  to root base +  $y$  to root base +  $x$  to root base +  $w$  to root base +  $v$  to root base +  $u$  to root base).

between two branching points (interior link), or between a free apex of the root (meristem) and the branching point closest to it (exterior link) was considered a root structural unit (Fitter, 1991; Fig. 1). A pathlength of an exterior link was defined as the number of links between that exterior link and the root at its point of union with the stem (hereafter root base) (Fig. 1). According to Fitter (1991), the altitude of a root system ( $a$ ) is the number of links of the longest pathlength, the magnitude ( $\mu$ ) is the total number of exterior links, and the total exterior pathlength of a root system ( $Pe$ ) is the sum of the pathlengths for all exterior links (Fig. 1). The topological index of the root systems of a group of plants was calculated as the slope ( $\beta_1$ ) of the linear regression of the logarithm of  $Pe$  on the logarithm of  $\mu$  (independent variable) or that of  $\log a$  on  $\log \mu$  (Fitter et al., 1991; Fitter, 1991).

For all field plants, diameter of the root base, root length from the root base to the distal end of the root, and root length from the root base up to its first nodule were measured with a gauge.

The position of nodules and nodulated lateral roots on the main root was registered. These positions were indicated by the number of the link carrying a nodule (or a lateral nodulated root) counted from the main root base to the root apex (apical meristem). The number of nodules per link on the main root and on lateral roots were registered. The frequency distribution of the number of nodules in the main and lateral root links was analysed. Five frequency classes of nodulation per link were determined: (1) absence of nodules, (2) 1–2 nodules, (3) 3–4 nodules, (4) 5–6 nodules, and (5) 7–8 nodules per link. Multilobed nodules were counted as one nodule (Wall and Huss-Danell, 1997).

### *Statistical analyses*

In order to estimate the spatial arrangement pattern of nodules, we calculated the dispersion coefficient ( $DC = \text{variance}/\text{mean}$ ) (Rabinovich, 1980) of the number of nodules per link on the main and secondary roots for the plants collected in the field. The negative binomial frequency distribution (Kolmogorov-Smirnov, i.e.: KS) model (Sokal and Rohlf, 1981) was applied to analyse whether nodule arrangement on roots was aggregated.

To determine treatment effects in experimental seedlings, Mann-Whitney U-Test (experiment 1) and one-way analysis of variance (experiment 2) ( $p < 0.05$ ) were applied. Six tubes from each treatment of experiment 1 were randomly selected to analyse the nodule distribution in roots, and to compare the nodulation of these plants with that of plants collected in the field. The nodule positions of the plants of the 6 tubes were previously compared among themselves by using Kruskal Wallis test ( $p < 0.05$ ), to make sure that they belonged to a homogeneous group. Mann-Whitney U-test was used to compare growth parameters of field plants and experimental plants. A KS test ( $p < 0.05$ ) was applied to compare the frequency distribution of positions of nodulated links of both groups of plants.

## **3. Results**

### *Root architecture of seedlings under natural conditions*

Data of size and nodulation of *D. trinervis* individuals collected at the study site are shown in Table 1. The dispersion coefficient of the number of nodules per root link on the main and lateral roots showed an aggregated arrangement ( $DC = 2.76$  and  $2.17$ , respectively).

Table 1. Mean values and standard deviation (SD) of growth and nodulation of 40 *Discaria trinervis* seedlings, growing under natural conditions

	Mean	SD
Number of shoot internodes	9.0	2.0
Shoot height (cm)	4.2	2.0
Root length (cm)	6.8	2.0
Root base diameter (mm)	1.0	0.3
Depth of the first nodule (cm)	1.5	0.6
Number of links in main root	21.0	7.0
Number of nodules on main root	5.0	2.0
Number of nodules on lateral roots	4.0	3.0

Table 2. Effect of soil depth inoculum (0-3, 3-6 and 6-9 cm) on size parameters and nodulation of 5-month old *Discaria trinervis* seedlings

	Soil depth 0-3 cm		3-6 cm		6-9 cm	
	Mean n = 40	SD* n = 40	Mean n = 41	SD n = 41	Mean n = 41	SD n = 41
Number of shoot internodes	9.0	3.0	9.0	3.0	9.0	3.0
Shoot height (cm)	11.2	4.3	11.1	4.1	11.7	4.1
Root length (cm)	9.3	2.5	9.5	2.8	9.9	2.4
Number of links in main root	20.0	7.0	18.0	6.0	21.0	6.0
Number of nodules on main root	0.4	0.7	0.4	0.8	0.5	0.8
Number of nodules on lateral roots	3.4	5.1	3.5	4.2	2.7	3.7
Shoots dry weight (mg)	10.1	10.8	10.5	6.9	12.6	6.3
Roots dry weight (mg)	5.6	6.4	6.4	3.9	7.9	3.7

\*SD: Standard deviation. Note: Row-wise comparisons yielded non-significant differences ( $p > 0.05$ ). One-way Anova test.

For the main roots 82% of the links did not have nodules, 14% had 1 or 2 nodules, and the remaining (4%) had 3 to 8 nodules (Fig. 2a). This distribution did not deviate significantly from a negative binomial model (KS = 9.21,  $p > 0.05$ ). On lateral roots the nodules were distributed over only 7% of the links (to a maximum of 4 nodules per link) (Fig. 2b). In this case the distribution did not fit a negative binomial model (KS = 0.925,  $p < 0.05$ ) in spite of the nodules being grouped in certain root zones.

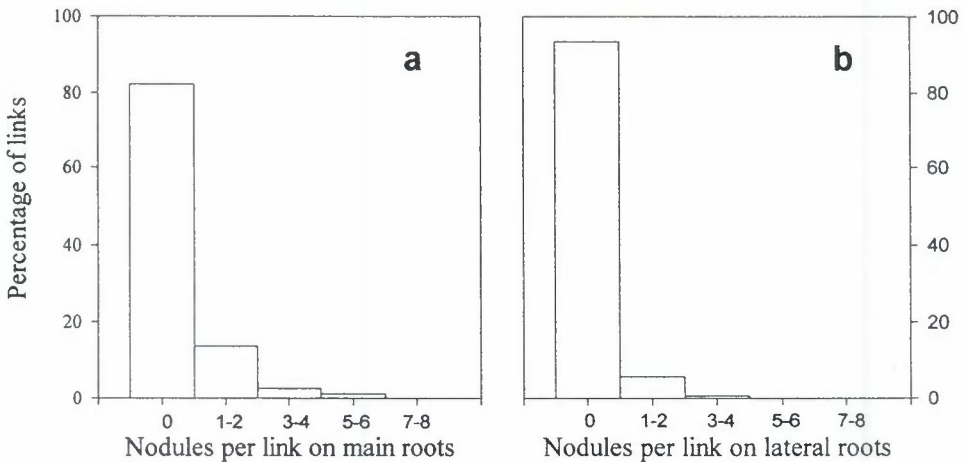


Figure 2. Frequency distribution of the number of actinorhizal nodules per link in 40 *Discaria trinervis* seedlings growing under natural conditions on (a) main roots, and (b) lateral roots.

The main root had nodules from the root base down to the 15th link and the lateral roots had most nodules from links 1 to 5 (Figs. 3a and 3e). Nodulated lateral roots were found between links 1 and 15 of the main root (Fig. 3c).

The topological index based on the logarithm of  $P_e$  vs  $\log \mu$  was 1.432 ( $p < 0.05$ ,  $R^2 = 95.5\%$ ), and that based on  $\log a$  vs  $\log \mu$  was 0.580 ( $p < 0.05$ ,  $R^2 = 65.6\%$ ). Both indices indicated a random type root branching model.

#### *Root architecture of seedlings under controlled conditions*

Experiment 1: all experimental plants growing in natural soil had actinorhizal and arbuscular mycorrhizal infection. Plants growing in tyndalized soil were not infected by *Frankia* or mycorrhizal fungi. Infected plants had longer shoots ( $U = 1365.0$ ,  $p < 0.05$ ) and more shoot internodes than uninfected plants ( $U = 1365.0$ ,  $p < 0.05$ ) suggesting the effectivity of the symbiosis.

The topological index for the infected plants showed a root branching model close to a random type (linear regression of  $\log a$  on  $\log \mu$ :  $Y = 0.605 + 0.456 x$ ,  $p < 0.05$ ,  $R^2 = 25\%$ ; and of  $\log P_e$  on  $\log \mu$ :  $Y = 0.811 + 1.193 x$ ,  $R^2 = 70.35\%$ ). The non infected plants, growing in the tyndalized soil, had a herringbone type topology model ( $\log a$  on  $\log \mu$ ,  $Y = 0.123 + 0.838 x$ ,  $p < 0.05$ ,  $R^2 = 77\%$ ). The shoot : root ratio (on length basis) was 0.5 for plants growing in tyndalized soil, and 1.8 for plants growing in non sterilised soil.



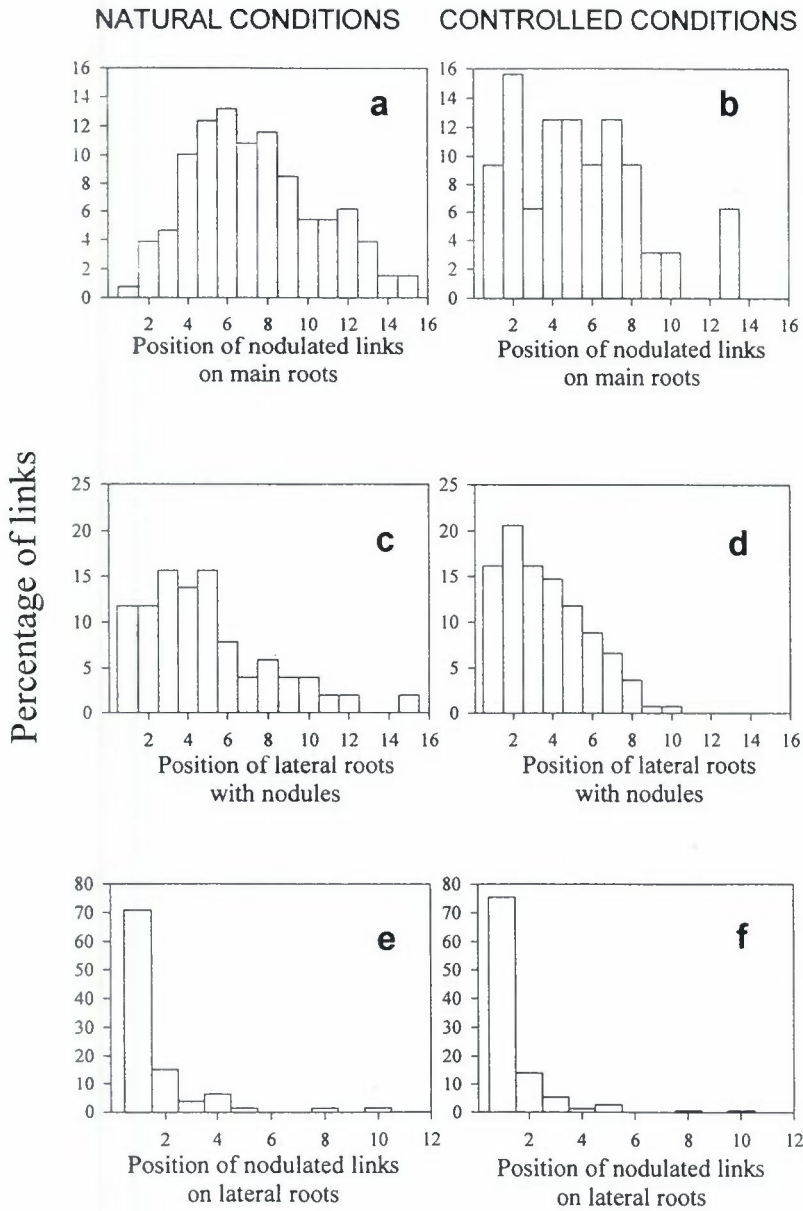


Figure 3. Position of nodules and lateral nodulated roots on the main roots in *Discaria trinervis* seedlings growing under natural (a, c, e) and controlled (b, d, f) conditions. Position is indicated by the number of the link carrying a nodule (or lateral nodulated root) counted from the root base to the apical meristem of the main root.

Experiment 2: all inoculated plants grown in soil from different depths were nodulated. Plants from different treatments did not show significant differences in root and shoot length, number of shoot internodes, number of main root links, dry weight of shoots and roots, and number of nodules ( $p > 0.05$ ) (Table 2). None of the negative control plants was nodulated.

Inoculated plants had nodules from link 1 to link 13 on the main root (Fig. 3b) and lateral nodulated roots appeared from link 1 to link 10 (Fig. 3d). Lateral roots had most nodules from link 1 to link 5 (Fig. 3f).

The number of shoot internodes and root links were similar between field plants and the randomly selected experimental plants ( $U = 1744.0$ ,  $U = 1791.0$ , respectively,  $p < 0.05$ ), the number of nodules on main roots was significantly higher for field plants than for plants from the nodulation experiment ( $U = 3039.5$ ,  $p < 0.05$ ). Field plants and experimental plants had similar frequency distributions of the positions of nodulated links in main roots (Figs. 3a, 3b), nodulated links in lateral roots (Figs. 3c, 3d) and lateral nodulated roots (Figs. 3e, 3f) ( $KS = 0.244$ ,  $KS = 0.247$ , and  $KS = 0.166$ , respectively,  $p > 0.05$ ).

#### 4. Discussion

The roots of *D. trinervis* plants from the field displayed a random topology. The random topology is an intermediate model between the herringbone topology, described for plants growing in resource-poor soils, and the dichotomous topology, characteristic of plants growing in more favourable soils (Fitter, 1991; Fitter, 1996). Fitter and Stickland (1991) also found a random topology for plants like *Plantago lanceolata*, *P. coronopus* and *Campanula persicifolia*, growing under controlled conditions. Since they grew in poor soils, *D. trinervis* plants would be expected to display a herringbone topology, that is more effective for exploring and exploiting the soil, particularly for the acquisition of mobile resources, such as nitrate ions (Fitter, 1996; Fitter and Stickland, 1991). However this root model was observed here only in experimental *D. trinervis* plants without root symbionts. Probably, the infection of *Frankia* and arbuscular mycorrhiza induces a different nutrient uptake strategy in *D. trinervis* plants. Although the fitness of the data of one of the linear regressions from the experimental plants is not good enough, due to the nature of the data set, we suggest that there is no contradiction with the predictions of the model, for two reasons. On one hand, for less mobile resources, such as  $H_2PO_4^-$ , topology is less important and these ions may be largely acquired by mycorrhizal hyphae (Fitter, 1996). On the other hand, *D. trinervis* plants in symbiosis with *Frankia* may obtain nitrogen from the atmosphere. According to Fitter (1991), the root topology of plants growing in

richer soils or where soil-based resources are less important, would tend to be closer to dichotomic.

*D. trinervis* nodules are arranged in groups on roots and there is a high percentage of links without nodules (Fig. 2). The nodulation assay performed here showed that this spatial pattern of root nodules is not a consequence of the distribution of infective units of *Frankia* in the soil. In *D. trinervis* seedlings, nodules are confined to some areas of the roots close to the base of the main root (Figs. 3a, 3b). The position of lateral nodulated roots agreed with the nodulated area of the main root (Figs. 3c, 3d). The same pattern of nodule position in plants from the field and experimental plants, suggests that the autoregulation of nodulation, described for *D. trinervis* seedlings inoculated and nodulated in pouches (Valverde and Wall, 1999b; Valverde et al., 2000), might be working in plants in their natural environment. The reduced shoot:root ratio found for non-nodulated plants reflected resource re-allocation within each plant to favour root development to capture nitrogen (Valverde et al., 2000; Lambers et al., 1998). As suggested by Valverde et al. (2000) autoregulation would be limiting the extension of the root where nodule formation is further allowed (Valverde et al., 2000).

The question that arises is how the regulation of the lateral root branching in plants with N<sub>2</sub> fixing nodules, which are themselves responding to a regulation mechanism (Wall and Huss-Danell, 1997; Valverde and Wall, 1999b; Valverde et al., 2000), is operating.

Studies on the regulation mechanisms on the symbioses of plants with *Rhizobium* or *Frankia* utilise either distance units around root tip position at the time of inoculation to measure nodule positions and construct nodulation profiles (Pierce and Bauer, 1983; Caetano-Anollés and Bauer, 1988; Burggraaf et al., 1983; Valverde and Wall, 1999b; Valverde et al., 2000), or percentage of nodule number to analyse nodule distribution (Wall and Huss-Danell, 1997). The present study provides a simple way to analyse the presence of nodules on roots, mainly for plants under natural conditions. The number of links showed a similar pattern for plants with similar growth parameters. Nodule position on a link number basis could be more suitable for the localisation of infectible cells on the root systems, than the use of distance units around the root tip, or percentage of nodule numbers. Furthermore, the modelling approach used here is a useful tool both to compare patterns of root branching and nodulation under natural and controlled conditions as a means of exploring the existence of mechanisms of control.

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#### REFERENCES

- Bianchi, E. 1987. Estudio ecológico de la Pampa de Huenuleo (San Carlos de Bariloche, Provincia de Río Negro) Parte I: Geomorfología. Cuadernos universitarios. Univ. Nac. del Comahue. Centro Reg. Bariloche.
- Burggraaf, A.J.P., Van Der Linden, J., and Tak, T. 1983. Studies on the localisation on infectible cells on *Alnus glutinosa* roots. *Plant and Soil* **74**: 175–188.
- Caetano-Anollés, G. and Bauer, W.D. 1988. Feedback regulation of nodule formation in alfalfa. *Planta* **175**: 546–557.
- Chaia, E.E. 1997. La simbiosis actinorrhízica en las Rhamnaceas del Parque y Reserva Nacional Nahuel Huapi. Doctoral Thesis. Universidad Nacional de La Plata, Argentina.
- Chaia, E. 1998. Isolation of an effective strain of *Frankia* from nodules of *Discaria trinervis* (Rhamnaceae). *Plant and Soil* **20**: 99–102.
- Fitter, A.H. 1991. The ecological significance of root system architecture: an economic approach. In: *Plant Root Growth, an Ecological Perspective*. D. Atkinson, ed. Oxford Blackwell Scientific Publications, London, pp. 229–243.
- Fitter, A. 1996. Characteristics and functions of root systems. In: *Plant Roots. The Hidden Half*. 2nd. Ed. Y. Waisel, A. Eshel, and U. Kafkafi, eds. Marcel Dekker Inc., New York, pp. 1–20.
- Fitter, A.H. and Stickland, T.R. 1991. Architectural analysis of plants root systems 2. Influence of nutrient supply on architecture in contrasting plant species. *New Phytologist* **118**: 383–389.
- Fitter, A.H. and Stickland, T.R. 1992. Architectural analysis of plant root systems III. Studies on plants under field conditions. *New Phytologist* **121**: 243–248.
- Fitter, A.H., Stickland, T.R., Harvey, M.L., and Wilson, G.W. 1991. Architectural analysis of plants root systems 1. Architectural correlates of exploitation efficiency. *New Phytologist* **118**: 375–382.
- Fontenla, S. 2000. Repercusión de las micorrizas en Patagonia, relación entre plantas hospedadoras y no hospedadoras. Doctoral Thesis. Universidad Nacional del Comahue. Argentina.
- Lambers, H., Chapin III, F.S., and Pons, T.L. 1998. *Plant Physiological Ecology*. Springer-Verlag, New York, 540 pp.
- Leyser, O. and Fitter, A. 1998. Roots are branching out in patches. *Trends Plant Science* **3**: 203–204.
- Muñoz, E. and Garay, A. 1985. Caracterización climática de la Provincia de Río Negro. INTA Estación experimental Región Agropecuaria de San Carlos de Bariloche. 58 pp.
- Oremus, P.A.I. 1980. Occurrence and infective potential of the endophyte of *Hippophae rhamnoides* L. ssp. *rhamnoides* in coastal sand-dune areas. *Plant and Soil* **56**: 123–139.

- Phillips, J.M. and Hayman, D.S. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158-161.
- Pierce, M. and Bauer, W.D. 1983. A rapid regulatory response governing nodulation in soybean. *Plant Physiology* **73**: 286-290.
- Rabinovich, J.E. 1980. Introducción a la Ecología de Poblaciones Animales. Consejo Nacional para la Enseñanza de la Biología, C.E.C.S.A., Mexico. 313 pp.
- Silvester, W.B., Balboa, O., and Martinez, J.A. 1985. Nodulation and nitrogen fixation in members of the Rhamnaceae (*Colletia*, *Retanilla*, *Talguenea* and *Trevoa*) growing in the Chilean matorral. *Symbiosis* **1**: 29-38.
- Sokal, R.R. and Rohlf, F.J. 1981. *Biometry. The Principles and Practice of Statistics in Biological Research*. 2nd. Ed. J. Wilson and S. Cotter, eds. W.H. Freeman and Company, New York, 859 pp.
- Tortosa, R.D. 1983. El género *Discaria* (Rhamnaceae). *Boletín de la Sociedad Argentina de Botánica* **22**: 301-335.
- Tortosa, R.D. and Cusato, M. 1991. Effective nodulation of rhamnaceous actinorhizal plants induced by air dried soils. *Plant and Soil* **131**: 229-223.
- Valverde, C. and Wall, L.G. 1999a. Time course of nodule development in *Discaria trinervis* (Rhamnaceae)-*Frankia* symbiosis. *New Phytologist* **141**: 345-354.
- Valverde, C. and Wall, L.G. 1999b. Regulation of nodulation in *Discaria trinervis* (Rhamnaceae)-*Frankia* symbiosis. *Canadian Journal of Botany* **77**: 1302-1310.
- Valverde, C., Wall, L.G., and Huss-Danell, K. 2000. Regulation of nodulation and nodule mass in relation to nitrogenase activity and nitrogen demand in *Discaria trinervis* (Rhamnaceae) seedlings. *Symbiosis* **28**: 49-62.
- Wall, L.G. and Huss-Danell, K. 1997. Regulation of nodulation in *Alnus incana*-*Frankia* symbiosis. *Physiologia Plantarum* **99**: 594-600.
- Zhang, H. and Forde, B.G. 1998. An *Arabidopsis* MADS box that controls nutrient-induced changes in root architecture. *Science* **279**: 407-409.
- Zhang, H., Jennings, A., Barlow, P.W., and Forde, B.G. 1999. Dual pathways for regulation of root branching by nitrate. *Proceedings of the National Academy of Science of the United States of America* **96**: 6529-6534.