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Infectivity of soilborne *Frankia* and mycorrhizae in *Discaria trinervis* along a vegetation gradient in Patagonian soil

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The infective capacities of the nitrogen fixing Actinomycete *Frankia* and arbuscular mycorrhizal fungi from soils near watercourses, along a vegetation gradient, were studied using plant bioassays. *Frankia* and arbuscular mycorrhizas capable of infecting *Discaria trinervis* were found at seventeen sites sampled. More specific enumeration of the infective capacities of both microorganisms in relation to environmental factors was performed in seven representative soils of the analysed vegetation zones (rainforest, xeric forest and steppe) using the most probable number method. The highest nodulation capacities ranged from 340 infective units g^{-1} soil, in a steppe marsh devoid of actinorhizas, to 61 in a coastal actinorhizal scrub (in xeric forest). The highest number of infective mycorrhizal units – also found in marsh – was 145. In general, rainforest soils had the lowest values for both microorganisms. Infective units of *Frankia* and arbuscular mycorrhizal fungi in soil were positively correlated ($r = 0.89$, $P < 0.05$). Both soilborne symbionts showed the highest infective capacity in semi-arid conditions nearby watercourse and at the valley bottom location. Tripartite symbiosis was effective in plants inoculated with steppe and xeric forest soils and plants inoculated with *Frankia* BCU110501 and *Glomus mosseae.* Interaction between both symbionts and influence of environmental conditions, in general, would contribute to define comparable trends of their infective capacities.

In northwestern Patagonia (Argentina), several native plant species belonging to the Rhamnaceae family (TORTOSA 1983) grow in different plant associations. Their root systems are naturally infected by the nitrogen fixing actinomycete *Frankia* forming an actinorhizal symbiosis (CHAIA 1997) and by arbuscular mycorrhizal fungi (AMF), which – in turn – form all-together a tripartite symbiosis (FONTENLA *et al.* 1998, FONTENLA *et al*. 2001). *Colletia hystrix* CLOS. occurs in forests and *Discaria* spp. in different environments on poor and gravel soils. *D. chacaye* (G. DON) TORT. grows along lake coasts, near forests and steppe sites. *D. trinervis* (HOOK et ARN.) REICHE grows in xeric forests and steppe sites, mainly along fresh water streams. *D. articulata* (PHIL.) MIERS occurs in ecotone and steppe areas. However, in some steppe sites the three species may co-exist. Some *Discaria* species are also present in disturbed sites, i.e. *D. trinervis* and *D. chacaye* grow in roadsides and abandoned fields. *D. articulata* resprouts in burned sites (RAFFAELE and VEBLEN 2001) and facilitates tree establishment (KITZBERGER *et al.* 2000). Senescent leaves of nodulated *D. trinervis* plants have a higher N content than other woody species in the Patagonian region and therefore, plants have a low N use efficiency (MAZZARINO *et al.* 1998) probably favouring major N contributions to the soil. The role of these plant species, with their sym-

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biotic relationships, in natural environments has not been established although they seem to be early-successional plants.

Frankia and AMF have a seasonal development in *D. trinervis* host plants, with higher *Frankia* infection rates during spring and summer (CHAIA 1993), and higher AMF infection rates during summer and autumn (FONTENLA, unpublished results). Experimental studies demonstrated that plant growth and nodule biomass proportion in *D. trinervis* are stimulated by *P* (VALVERDE *et al*. 2002), which may be acquired directly by root exploration of the soil or by AMF in field plants (FOX *et al.* 1990).

Frankia and AMF have a wide occurrence in different soils and may be absent under particular conditions (VARMA 1995, ZIMPFER *et al.* 1997, HUSS-DANELL *et al.* 1999, WIJNHOLDS and YOUNG 2000, BATZLI *et al.* 2004). However, relatively few are known about the infective capacities of soilborne *Frankia* and AMF in native *Discaria* species.

To better understand the importance of tripartite symbiotic association under natural conditions in Patagonian ecosystems, *Frankia* and AMF infective capacities from different soils along a vegetation gradient were analysed. Tripartite symbiosis effectivity was also evaluated in *D. trinervis* plants inoculated with soils or with pure microorganisms' strains.

Materials and methods

Study area: Research was conducted in an area located in the Andean and Extra-Andean region in northwestern Patagonia at 760–800 m elevation. This area included a variety of vegetation units between the rainforest (41°05′ S, 71°49′ W) and the semi-arid steppe (40°41′ S, 71°01′ W) (Fig. 1). Climate is temperate cool, with a steep precipitation gradient in only 50–100 km eastward, from 3000 to 700 mm yr^{-1}, and predominant west-east winds (BARROS *et al*. 1983). Soils are Andisols in rainforest (Andean region), which have been originated in volcanic ashes (MAZZARINO *et al*. 1998) whereas in xeric forest and steppe sensu lato (Extra-Andean region) are Mollisols and Aridisoles. The area also comprised two wetland azonal soils, a marsh (a steppe azonal site flooded in autumn and winter) and a waterlogged peat bog (mosaic of water-retaining *Sphagnum* prairies) (ROIG 1998).

A total of seventeen sites were established near watercourses or flooded areas (Fig. 1) with or without actinorhizal shrubs. Fifteen sites were located inside a more pristine area, the Parque y Reserva Nacional Nahuel Huapi (Nahuel Huapi National Park and Reserve), and two other nearby sites. A complete description of each site is presented in Table 1.

Soil and vegetation sampling and analysis: Three soil samples per site (about 500 g each) were randomly collected in summer at 0 to 15 cm depth from under Rhamnaceae plants, if present, or under the dominant plant species. Soils were collected in the same sampling instance in two subsequent days. Samples taken from the same site were combined. Soil samples were stored moist at 4 °C, for further use in plant bioassays, or air-dried and stored at room temperature for chemical analyses. There was no variation in the storage time between the different soil samples and all the samples were treated in the same way.

Actinorhizal nodulation presence in *Discaria* spp. plants was recorded in at least three plants per site. *Discaria* spp. seeds were collected and dry-stored at –20 °C.

Soil properties were determined with the following techniques: pH in H_2O (THOMAS 1996), extractable P (KUO 1996), electrical conductivity (EC) (BLAKEMORE *et al.* 1987), exchangeable potassium (K) (KNUDSEN *et al.* 1982), exchangeable magnesium plus calcium (Mg + Ca) (LANYON and HEALD 1982). Total N determinations were performed by semi-micro KJELDAHL method (BREMNER 1996) and the organic carbon (C) percentage was determined following NELSON and SOMMERS (1996).

Plant bioassays: *D. trinervis* seeds were scarified (GAUTHIER *et al.* 1984), and germinated on sterile humid vermiculite.

To test *Frankia* and AMF propagule occurrence in soils from the whole study area, four seedlings at the cotyledon stage were transferred to 75 ml pots filled with a mixture (1 : 1 v/v) of tyndalized soil and vermiculite (open flow in autoclave for 1 h, for 3 consecutive days) as substrate (Experiment 1). Pots were inoculated with 5 g of each soil sample. Positive controls were inoculated with *Frankia* strain BCU110501 (CHAIA 1998) and *Glomus mosseae* (soybean rhizosphere soil). Twelve pots with

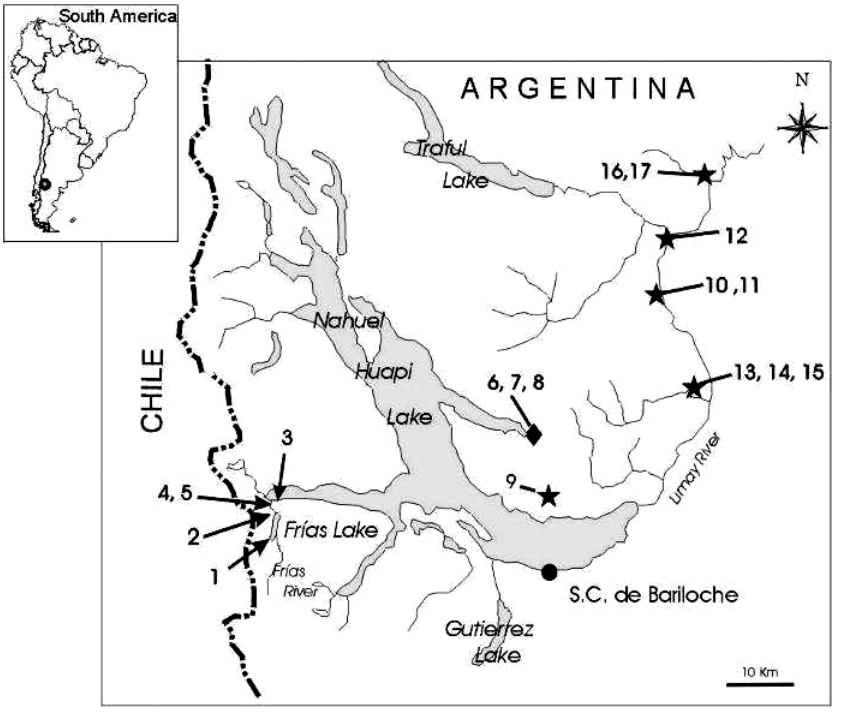

Fig. 1
Schematic map of the sampling sites (location) along a northwestern Patagonia vegetation gradient. Left to right ordered numbers indicate sites in close proximity. All sites, except 16 and 17 are in the Parque y Reserva Nacional Nahuel Huapi. (►) Rainforest sites: (1) coastal forest (Puerto Frías); (2) coastal mixed scrub (Puerto Alegre); (3) coastal *D. chacaye* scrub (Puerto Blest); (4) waterlogged peat bog (Puerto Blest); (5) bamboo thicket (Puerto Blest). (♦) Xeric forest sites (Península Huemul): (6) forest; (7) scrub transitional with forest; (8) coastal *D. chacaye* scrub. (★) Steppe sites: (9) marsh (Tequel Malal); (10) shrubby steppe along river (7 Cóndores); (11) herbaceous steppe (Valle Encantado); (12) shrubby steppe, with trees (Confluencia); (13) gallery forest along river, with *D. trinervis* (Bandurrias); (14) gallery forest along river, with *D. chacaye* (Bandurrias); (15) herbaceous steppe, with *D. chacaye* (Bandurrias); (16) herbaceous steppe, with shrubs (Montura Chilena); (17) gallery forest along temporary stream (Montura Chilena)

seedlings were also inoculated with 5 g of a tyndalized mixture from the seventeen soils to serve as negative controls. Assay had four replicates per treatment. Plants were fertilised during the first week with N and P-free HOAGLAND's nutrient solution diluted to one fourth of full strength (TORTOSA and CUSATO 1991). Until the end of the experiment, plants were watered with tap water as necessary.

The quantification of *Frankia* and AMF infective units (IU) of seven soils was performed by using the most probable number method (MPN) (HALVORSON and ZIEGLER 1933, WOOMER 1994) (Experiment 2). These soils represented different environmental conditions of vegetation zones. Sites were selected according to: a) plant association; b) results of Experiment 1; and c) soil homogeneous groups, mainly representing soil chemical properties (see below Cluster Analysis). Each soil sample was prepared by externally kneading it by hand and then sieving it (3 mm mesh). Seven successive 5-fold dilutions (5^{-1} to 5^{-7}) were prepared directly diluting and thoroughly mixing each soil sample (on a dry weight basis) with a sterile sand and vermiculite mixture (1 : 1 v/v). A 30 ml sample from each respective soil dilution was placed in a sterile glass tube and watered with HOAGLAND's nutrient solution diluted to one fourth of full strength, with 0.07 mM N as NH_4NO_3 and 0.1 mM P as KH_2PO_4. Two *D. trinervis* seedlings at the cotyledon stage were transferred to each tube. There were six replicates for each dilution. Twenty non-inoculated tubes were used as contamination controls. Plants

Table 1
Description of sampling sites

Vegetation unit[1] and site description (site number)[2]	Dominant and main subordinate plants,[3] **Actinorhizal species**	Rainfall[4] (mm yr^{-1})
Rainforest		
Coastal forest (1)	*Nothofagus dombeyi*, *Weinmannia trichosperma*, *Fitzroya cupressoides*	2500
Coastal mixed scrub (2)	*Chusquea culeou*, *Azara lanceolata*, *Berberis linearifolia*, *Nothofagus dombeyi*	2500
Coastal *D. chacaye* scrub (3)	***Discaria chacaye***, *Desfontainea spinosa*, *Fuchsia magellanica*	3000
Waterlogged peat bog (4)	*Pilgerodendron uviferum*, *Fitzroya cupressoides*, *Nothofagus dombeyi*, *Sphagnum magellanicum*	3000
Bamboo thicket (5)	*Chusquea culeou*, *Schinus patagonica*, *Nothofagus antarctica*, *Berberis buxifolia*	3000
Xeric forest		
Forest (6)	*Austrocedrus chilensis*, *Lomatia hirsuta*, *Ovidia andina*, ***Colletia hystrix***	1800
Scrub transitional with forest (7)	*Maytenus chubutensis*, *Lomatia hirsuta*, *Schinus patagonica*, *Austrocedrus chilensis*, ***Discaria chacaye***	1800
Coastal *D. chacaye* scrub (8)	***Discaria chacaye***, *Schinus patagonica*, *Rosa rubiginosa*	1800
Steppe		
Marsh (9)	*Juncus arcticus*, *Taraxacum officinale*, species of Poaceae and Cyperaceae	1000
Shrubby steppe along river (10)	***Discaria articulata***, *Mulinum spinosum*, *Berberis buxifolia*, *Stipa speciosa*, ***Discaria trinervis***	1000
Herbaceous steppe (11)	*Stipa speciosa*, *Mulinum spinosum*, ***Discaria trinervis***, ***Discaria chacaye***, ***Discaria articulata***	1000
Shrubby steppe, with trees (12)	*Stipa* spp., ***Discaria articulata***, *Mulinum spinosum*, *Austrocedrus chilensis*, ***Discaria trinervis***, ***Discaria chacaye***	900
Gallery forest along river, with *D. trinervis* (13)	***Discaria trinervis***, *Nothofagus antarctica*, *Maytenus boaria*, ***Discaria chacaye***	800
Gallery forest along river, with *D. chacaye* (14)	***Discaria chacaye***, *Salix fragilis*, *Maytenus boaria*, *Berberis darwinii*, ***Discaria trinervis***	800
Herbaceous steppe, with *D. chacaye* (15)	*Stipa speciosa*, *Mulinum spinosum*, ***Discaria chacaye***, *Senecio filaginoides*	800
Herbaceous steppe, with shrubs (16)	*Stipa speciosa*, *Mulinum spinosum*, *Schinus patagonica*, ***Discaria chacaye***, ***Discaria articulata***	700
Gallery forest along temporary stream (17)	***Discaria chacaye***, ***Discaria trinervis***, *Maytenus boaria*, *Berberis buxifolia*, ***Discaria articulata***	700

[1] Vegetation was characterized following ROIG (1998) and MERMOZ and MARTIN (1986)
[2] See site localities in Fig. 1
[3] Nomenclature follows EZCURRA and BRION (2005)
[4] Mean annual rainfall (BARROS *et al.* 1983)

were weekly fertilised with 2 ml of the HOAGLAND's solution without P and N salts. The plants inoculated with the 5^{-1} soil dilution were used to compare the symbiosis effectiveness.

Tripartite symbiosis synthesis in *D. trinervis* plants was done with strains of both microorganisms (Experiment 3). Two seedlings were transferred to each sterile glass tube filled with a sand and vermiculite mixture (1 : 1 v/v) inoculated with *Frankia* strain BCU110501 (CHAIA 1998) or with 5 g of soil containing *Glomus mosseae* (soybean rhizosphere soil), or with both microorganisms together.

Non-inoculated plants were used as negative controls. Assay had five replicates per treatment. Fertilization was carried out as in Experiment 2.

All plants from the three experiments were cultivated in a growth chamber for twelve weeks, with 16 hs photoperiod (photosynthetic photon flux density ca. 70 μM m^{-2} s^{-1}) at 19–22 °C, and 24% relative humidity. Nodulation and AMF infection were recorded. Each replicate root sample was fixed in 70% ethanol, cleared and stained following PHILLIPS and HAYMAN (1970). In plants of Experiment 1 AMF infection was evaluated using the visual method, a root scanning non-systematic procedure (SCHENCK 1982). Stained roots were uniformly spread in a Petri dish, the whole sample was carefully rotated on the microscope stage, and an assessment of the colonized roots was done estimating three colonization percentage categories, namely: low (less than 30%); moderate (between 30 and 70%); and high (more than 70%). Within a sample, the percentage of root with mycorrhizae was determined establishing the ratio between the number of feeder roots colonized by AMF fungi (arbuscules, vesicles and/or hyphae) and the total feeder roots.

Plant growth (shoot dry mass and shoot: root length ratio) and AMF infection percentage were recorded in every plant inoculated with the 5^{-1} soil dilution from the Experiment 2 and in plants from Experiment 3. AMF infection percentage in these two experiments was evaluated using a gridline-intersect method (GIOVANNETTI and MOSSE 1980).

Data analysis: In order to organize all the sites from the whole study area according to soil homogeneous groups they were classified by performing hierarchical cluster analysis following WARD method (LEBART *et al.* 1997). Classification of sites according to actinorhizal plants presence (Table 1) and soil chemical properties (pH, C, N, EC, Table 2), resulted in four clusters with

Table 2
Soil chemical analysis

Vegetation unit and site description (site number)	Soil characteristics						
	pH	EC (μS cm^{-1})	C (%)	N (%)	P (ppm)	K (meq 100 g^{-1})	Ca + Mg (meq 100 g^{-1})
Rainforest							
Coastal forest (1)	6.0	33,4	6.9	0.33	na[1]	na	na
Coastal mixed scrub (2)	4.8	65.7	14.3	0.44	na	na	na
Coastal *D. chacaye* scrub (3)	5.9	47.2	2.5	0.18	2.4	0.20	3.9
Waterlogged peat bog (4)	5.3	137.0	33.7	0.67	1.9	0.53	9.3
Bamboo thicket (5)	5.3	174.0	37.9	0.93	3.7	0.52	5.7
Xeric forest							
Forest (6)	5.9	36.1	6.0	0.41	na	na	na
Scrub transitional with forest (7)	6.6	19.3	2.5	0.14	na	na	na
Coastal *D. chacaye* scrub (8)	6.1	24.3	2.0	0.16	6.2	0.44	9.7
Steppe							
Marsh (9)	6.0	56.8	5.4	0.42	4.1	0.66	11.1
Shrubby steppe along river (10)	6.8	14.4	1.4	0.09	na	na	na
Herbaceous steppe (11)	6.8	39.0	1.7	0.09	6.7	0.77	13.2
Shrubby steppe, with trees (12)	6.5	27.8	2.2	0.16	na	na	na
Gallery forest along river, with *D. trinervis* (13)	6.7	53.2	1.8	0.13	na	na	na
Gallery forest along river, with *D. chacaye* (14)	6.5	63.7	1.8	0.17	na	na	na
Herbaceous steppe, with *D. chacaye* (15)	5.7	37.2	1.5	0.11	na	na	na
Herbaceous steppe, with shrubs (16)	6.8	21.8	1.1	0.10	na	na	na
Gallery forest along temporary stream (17)	6.8	50.7	2.2	0.07	8.8	1.65	18.8

[1] na: not analyzed

increasing EC values from cluster I to IV. Cluster I included all sites from the xeric forest, most steppe sites excluding the marsh and gallery forests, and the coastal forest site (rainforest). Cluster II included strongly watercourse associated sites: all gallery forest sites (steppe), two coastal rainforest sites (coastal mixed scrub and coastal *D. chacaye* scrub) and the marsh. Clusters III and IV included rainforest azonal sites: the waterlogged peat bog and the bamboo thicket, respectively. The latter differed notably from the others regarding soil characteristics. They had the lowest pH and the highest EC, C, and N values (Table 2).

Data from Experiments 2 and 3 were calculated as average per plant within each tube. Confidence intervals ($P < 0.05$) were applied to compare *Frankia* and AMF IU per soil gram (WOOMER 1994). Plant growth parameters, nodule number per plant, and AMF infection percentage in inoculated 5^{-1} soil dilution *D. trinervis* seedlings were compared by one-way ANOVA. Differences between pairs of means were assessed by Scheffé's procedure ($P < 0.05$). Shoot dry mass and AMF percentage data were square root transformed, and the number of nodules per plant was transformed as $1/(x+\sqrt{2})$ to satisfy statistical requirements. Reciprocal transformation was applied to shoot:root ratio. For Experiment 3, shoot dry mass and shoot : root ratio were analysed as before. Number of nodules per plant and AMF infection percentage was compared using KRUSKAL-WALLIS and DUNN's tests. SPEARMAN's correlations were performed between *Frankia* IU, AMF IU, number of *Frankia* nodules per plant, and AMF infection percentage in 5^{-1} soil dilution inoculated plants (see Experiment 2). These variables were also correlated with the soil chemical characteristics, the rainfall and the presence or absence of actinorhizal plants in every site (ZAR 1999).

Results

As described in Table 2, all soils examined had a slightly acid pH, with the highest values in xeric forest and steppe sites (5.9 to 6.8), and the lowest in most rainforest sites. All the rhamnaceous registered plants in the study area were nodulated. The fact that the soil samples led to a tripartite symbiosis was the evidence for the presence of *Frankia* and AMF propagules. Soils from the waterlogged peat bog, the bamboo thicket and the marsh produced nodulation in 50 to 60% test plants. Soils from the first two sites and from the coastal mixed scrub (rainforest) produced low AMF infective values in *D. trinervis* plants (Table 3). Negative controls were never infected with *Frankia* or AMF. Positive controls were always nodulated and AMF infected.

Frankia and AMF IU values differed among sites ($P < 0.05$) (Table 4). *Frankia* IU highest values were found in two steppe soils (marsh and gallery forest along temporary stream) and in xeric forest site (coastal *D. chacaye* scrub). The lowest value was found in the bamboo thicket soil. Used soil dilutions did not allow any *Frankia* propagules detection in the waterlogged peat bog soil. AMF IU could be separated in three significantly different groups ($P < 0.05$). The highest value was found in the marsh (145 IU) and the intermediate in the gallery forest along a temporary stream. The three tested soils from the rainforest, the coastal *D. chacaye* scrub in the xeric forest and the herbaceous steppe (from Valle Encantado) had the lowest AMF IU values (Table 4).

D. trinervis plants inoculated with the first soil dilution series differed in plant growth and infection at harvest (Table 4). Steppe and xeric forest soils inoculated plants had a higher shoot dry mass than rainforest soils inoculated plants ($F = 31.28$, $P < 0.01$). Shoot : root length ratio was higher in marsh and other steppe and xeric forest soils inoculated plants than in rainforest inoculated ones ($F = 13.56$, $P < 0.01$). The number of nodules per plant was higher in plants inoculated with soils from steppe, xeric forest, and rainforest coastal *D. chacaye* scrub than in all other soils inoculated plants ($F = 152.82$, $P < 0.01$). AMF infection intensity was lower in waterlogged peat bog soil inoculated plants than in all other plants ($F = 20.00$, $P < 0.01$) (Table 4).

The correlation matrix showed that *Frankia* IU were positively correlated with AMF IU ($r = 0.89$, $P = 0.007$), and with AMF plant infection ($r = 0.79$ $P = 0.036$). The AMF IU were positively correlated with number of nodules ($r = 1.00$, $P < 0.001$) and soil pH ($r = 0.76$,

$P = 0.046$), and negatively correlated with rainfall ($r = -0.90$, $P = 0.006$). Number of nodules per plant was also positively correlated with soil pH, and negatively with rainfall ($r = -0.90$, $P = 0.006$). The AMF plant infection was positively correlated with number of nodules ($r = 0.79$, $P = 0.036$), with soil pH ($r = 0.78$, $P = 0.038$), and with soil P ($r = 0.89$, $P = 0.006$); and negatively with rainfall ($r = -0.84$, $P = 0.017$). Correlations between other pairs of variables were not significant at $P < 0.05$.

The pairs AMF IU – AMF infection percentage, and *Frankia* IU – number of nodules per plant were not considered.

D. trinervis seedlings inoculated with *Frankia* BCU110501 and with *Frankia* + *G. mosseae* had a higher shoot dry mass than plants only inoculated with the AMF fungi, and the negative controls ($F = 43.74$, $P < 0.01$). All inoculated plants had a higher shoot : root ratio than the negative controls ($F = 18.31$, $P < 0.01$). A similar number of nodules was produced in plants inoculated only with *Frankia* and in plants inoculated with *Frankia* + *G. mosseae* ($H = 16.4$, $P = 0.01$). AMF highest infection percentage was registered in plants inoculated with both microorganisms simultaneously ($H = 16.1$, $P = 0.001$) (Table 5).

Discussion

Actinorhizal nodules and AMF have been found in all plants of the Rhamnaceae family observed so far in NW Patagonia (CHAIA 1997, FONTENLA 2000) and these symbioses could be experimentally reproduced with *D. trinervis* test plants, to show the infective capacity of soilborne *Frankia* and AMF propagules from different sites.

Table 3
Nodulation and mycorrhization response of *D. trinervis* test plants to inoculation with all soil samples from the various vegetation units

Vegetation unit and site description (site number)	Nodulation[1]	AMF[2]
Rainforest		
Coastal forest (1)	8/8	2
Coastal mixed scrub (2)	16/16	1
Coastal *D. chacaye* scrub (3)	16/16	3
Waterlogged peat bog (4)	7/14	1
Bamboo thicket (5)	10/15	1
Xeric forest		
Forest (6)	12/12	3
Scrub transitional with forest (7)	15/16	3
Coastal *D. chacaye* scrub (8)	12/12	2
Steppe		
Marsh (9)	10/15	2
Shrubby steppe along river (10)	16/16	2
Herbaceous steppe (11)	10/10	2
Shrubby steppe, with trees (12)	11/12	3
Gallery forest along river, with *D. trinervis* (13)	16/16	3
Gallery forest along river, with *D. chacaye* (14)	16/16	2
Herbaceous steppe, with *D. chacaye* (15)	16/16	2
Herbaceous steppe, with shrubs (16)	16/16	2
Gallery forest along temporary stream (17)	16/16	3

[1] Nodulated seedlings/number of tested seedlings
[2] Infection levels categories of arbuscular mycorrhizal fungi (AMF) (0: without infection; 1–3: infection levels categories)

Table 4
Infectivity (IU g^{-1} soil) and effectivity of soilborne *Frankia* and mycorrhizae (AMF) in *D. trinervis* from representative sites of a vegetation gradient

Vegetation unit and site description (site number)	Soil infective capacity[1]		Mean plant growth and infection[2]			
	Frankia (IU g^{-1} soil)[4]	AMF (IU g^{-1} soil)	Shoot dry mass (mg)	Shoot : Root (length)	Nodules per plant	AMF[3] (%)
Rainforest						
Coastal *D. chacaye* scrub (3)	25.0$^{cd\ 5}$	1.7^{a}	7.1^{a}	1.0^{ab}	6.3^{b}	32.7^{b}
Waterlogged peat bog (4)	0.0^{a}	0.6^{a}	7.7^{a}	0.8^{a}	0.0^{a}	1.3^{a}
Bamboo thicket (5)	0.8^{b}	1.7^{a}	7.8^{a}	0.7^{abc}	0.1^{a}	50.3^{bc}
Xeric forest						
Coastal *D. chacaye* scrub (8)	61.0^{de}	3.3^{a}	25.6^{b}	1.2^{cd}	7.0^{b}	81.3^{c}
Steppe						
Marsh (9)	340.0^{e}	145.0^{c}	29.6^{b}	1.3^{d}	11.8^{b}	79.8^{c}
Herbaceous steppe (11)	8.5^{c}	3.5^{a}	22.3^{b}	1.1^{bcd}	9.4^{b}	63.0^{bc}
Gallery forest along temporary stream (17)	98.0^{de}	23.0^{b}	25.8^{b}	1.3^{d}	9.9^{b}	85.2^{c}

[1] Data compared by Confidence intervals
[2] Plants inoculated with soil-diluted 1 : 5 v/v. Data compared by One-way ANOVA and SCHEFFÉ's tests ($n = 6$)
[3] AMF infection percentage following GIOVANNETTI and MOSSE (1980)
[4] Infective units per soil gram
[5] Different letter codes (a, b, c, d, e) within a column represent significant differences ($P < 0.05$)

It is known that *Frankia* infective capacity of soils could be largely controlled by the physiological status of *Frankia* (MYROLD and HUSS-DANELL 1994), and by vegetation types, that could favour *Frankia* saprophytic growth (MAUNUKSELA *et al.* 1999).

The presence or absence of actinorhizal plants in all NW Patagonian studied sites did not affect *D. trinervis* – *Frankia* soil infective capacity, considering the lack of correlation between these variables. These results are in accordance with previous studies that have shown a wide occurrence of *Frankia* propagules in different soils, including those lacking host plants (HUSS-DANELL and FREJ 1986, ZITZER and DAWSON 1992, ZIMPFER *et al.* 1997, WIJNHOLDS and YOUNG 2000). It is possible that the relatively short distance along the whole study area (about 50 km) and non-very extreme environmental conditions could be factors that may have influenced propagules' dispersion and formation.

Table 5
Mean growth and infection in *D. trinervis* inoculated with *Frankia* BCU110501 and *Glomus mosseae* ($n = 5$)

Inocula	Shoot dry mass[1] (mg)	Shoot : root[1]	Nodules[2] per plant	AMF[2] (%)
Frankia + AM	26.2$^{b\ 3}$	1.2^{b}	6.8^{b}	43.4^{b}
Frankia	22.1^{b}	1.2^{b}	6.6^{b}	0.0^{a}
AM	9.1^{a}	0.8^{b}	0^{a}	3.2^{a}
Negative control	5.3^{a}	0.6^{a}	0^{a}	0.0^{a}

[1] Data compared by One-way ANOVA and SCHEFFÉ's tests
[2] Data compared by KRUSKAL-WALLIS and DUNN's tests
[3] Different letter codes (a, b) within a column represent significant differences ($P < 0.05$)

In this study, the highest absolute *D. trinervis*-infective *Frankia* IU was found in the marsh, an azonal steppe site with temporary flooding, devoid of actinorhizal plant species. Most sites located near watercourses presented significantly high or intermediate values, with a decreasing trend along the vegetation gradient from the steppe to the xeric forest and to the coastal *D. chacaye* scrub in the rainforest. The herbaceous steppe, a site with dry conditions and without watercourses, had lower *Frankia* IU than other steppe sites. BATZLI *et al.* (2004) found that soilborne *Frankia* strains from a sand dune differ in their nodulation capacities along ecological gradients. The authors reported that soil infectious capacity declined with increasing levels of soil moisture, where submerged soils had no infectious capacity and soils with greater in situ moisture content or subject to intermittent saturation, tended to have lower infectious capacities. Our results from steppe and xeric forest sites also seem to suggest the existence of a gradient in *Frankia* infectious capacity, but in a different sense. The infective capacity was favoured by proximity to watercourses or to temporary flooding under semi-arid conditions. *Frankia* infective capacity of soils from rainforest sites showed a different behaviour than in drier sites, which was in agreement with the trends described by BATZLI *et al*. (2004). Both, the bamboo thicket and the waterlogged peat bog, with high precipitation levels that would contribute to maintain high soil moisture, had low or even no detectable infective values. Furthermore, they had particular vegetation, but similar soil characteristics, such as low pH, high EC, and C : N ratios.

River and lake waters could be relevant *Frankia* dispersal agents as proposed in other studies (ARVEBY and HUSS-DANELL 1988, HUSS-DANELL *et al.* 1997). This suggestion is supported by the presence of infective *Frankia* propagules in Nahuel Huapi lake sediments (CHAIA *et al*. 2005), where some coastal sites of the present study were located.

The mycorrhizal infective capacity of soils showed similar trends to those displayed by *Frankia.* AMF highest IU was found in marsh soil, and plants inoculated with this soil had a high AMF infection. AMF intermediate IU values decreased from the steppe site along a temporary stream to the xeric forest. AMF high values' infective capacity was associated, once again, with humid soils neighbouring drier environmental conditions. The lowest values were found in rainforest sites, in agreement with VARMA (1995), who also described low soil infectivity with high moisture levels.

Steppe and xeric forest soils sampled in this study produced effective tripartite symbiosis with *D. trinervis* seedlings (higher shoot dry mass than non-nodulated plants inoculated with rainforest soils), in coincidence with high and intermediate values of infective propagules in these soils. On the contrary, plants inoculated with rainforest coastal *D. chacaye* scrub (Puerto Blest) or bamboo thicket soils were nodulated and AMF infected, but apparently the symbiosis was not effective (similar shoot dry mass to non-nodulated plants inoculated with the waterlogged peat bog soil, similar also to non-inoculated plants). Differences found in the growth of plants inoculated with soils could be attributed to different factors. *Frankia* and AMF symbionts play an important role favouring the plant growth, but other soil microorganisms interacting in the rhizosphere could also have stimulating or inhibiting effects (PROBANZA *et al*. 1997).

An effective tripartite symbiosis could be also formed *in vitro* in *D. trinervis* plants inoculated with *Frankia* and AMF pure inocula. Similar shoot dry mass between *Frankia* alone and *Frankia* + AMF treatments, suggests the qualitative importance of the *Frankia* infection in tripartite symbiosis effectivity for the *in vitro*, mainly N limited, growth conditions. The finding that there was a positive correlation between AMF plant infection and the number of nodules in the experiments with soils, strongly suggests an interaction between both symbioses, probably because of physiological complementation at the nutrient level. N supplied by the *Frankia* N_2 fixation to the host plant would stimulate mycorrhizal infection (BRUNDRETT 1991), while at the same time, P supply by AMF would stimulate nodule development by *Frankia* symbiosis (VALVERDE *et al*. 2002), creating together a positive feed back in both symbiosis' development. Mycorrhizal infection would also contribute to a successful

actinorhizal symbiosis, especially in P-deficient soils, supposedly by improving mineral nutrition and protection against environmental stress (SCHWENCKE and CARÚ 2001).

In conclusion, soils from vegetation gradient sites could establish tripartite symbiosis. Semi-arid conditions and proximity to watercourses were the most suitable favouring the infective capacity of *Frankia* and AMF in soils. In general, interaction between both symbionts and influence of environmental conditions along the wide vegetation gradient in Patagonia, would define *Frankia* and AMF comparable trends of infective capacities, although we found some exceptions to this general rules that suggest an independency between the quality of symbionts' distribution among the different studied sites, specially within rainforest and xeric forest. This study highlights the fact that the microsymbionts that benefit some pioneering plants growth' find better conditions to infect host plants in semiarid environments, which are those highly susceptible to be affected by desertification processes.

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