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Occurrence of *Discaria trinervis* nodulating *Frankia* in dated sediments of glacial Andean lakes

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Abstract
Short sediment cores of four glacial Andean lakes were used to assess the occurrence of *Discaria trinervis*-infective *Frankia* by performing plant bioassays. Unperturbed sediment cores, collected at 20 to 400 m distance from shorelines were sub-sampled, lyophilized and dated by the ^{210}Pb and ^{137}Cs techniques. The change in infectious capacity of lyophilized samples was determined by comparing plants inoculated with air-dried soils from lakeshores with plants inoculated with a subsample of the respective lyophilized soil. Nodulation capacity was similar for soils treated by the two procedures. Superficial sediments of the four lakes and subsurface sediments of two of the tested lakes caused nodulation in *D. trinervis*, which could be mostly observed after 4 to 8 weeks after of inoculation time. The long distances between sites of core collections and lakeshores, and the water depth variation of sampling points (10 to 42 m depth), would suggest that *Frankia* infective propagules are transported in water. Water movements during the mixing periods would allow *Frankia* propagules removal from superficial sediments allowing dispersion to other shores. The estimated age of sediments indicated that *Frankia* could retain infectivity up to ca. 50 yrs.

Keywords: *Frankia*, *Discaria* spp., sediments, Andean lakes, nodulation capacity

1. Introduction

Actinorhizal plants occurring in coastal soils interact with both the terrestrial and the aquatic ecosystems. It is known that *Alnus* and *Myrica* bordering coasts of lakes and streams are a significant source of nitrogen, through drainage of leachates from direct leaf-fall or release during decomposition of foliage nearby these aquatic environments (Wetzel, 2001). Waters also serve as a dispersal agent for the nitrogen fixing endosymbiont *Frankia* (Arveby and Huss-Danell, 1988; Huss-Danell et al., 1997). Huss-Danell et al. (1997) suggested that *Frankia* could enter in the waters after being released in the soil by the broken senescent actinorhizal nodules. These authors have shown that superficial lake and river sediments in Northern Sweden and in Alaska contained infective and effective *Frankia*, which could be transported in water, thus providing opportunities to infect *Alnus* plants in the shores.

The nodulation of *Alnus tenuifolia* plants growing in sediment bars of an Alaskan river would support this assumption (Huss-Danell et al., 1997). Moreover, Zitzer and Dawson (1992) proposed that the high infectivity levels in *A. glutinosa* and *Elaeagnus angustifolia* produced by alluvial soils in Illinois could be due to deposition of sediments carried by floods, which could include organic matter, nutrients, and possibly *Frankia* propagules.

In the North West Patagonian region (Argentina), several native plant species belonging to the family Rhamnaceae (Tortosa, 1983), grow in different plant associations, with their root systems naturally infected by *Frankia* (Chaia, 1997). *Discaria* spp. occur mostly in scrubs in poor soils and on gravelly soils along freshwater courses, i.e. *D. trinervis* usually grows along streams and *D. chacaye* along lake coasts. *D. articulata* grows in semi-arid environments. In many scrublands or in gallery forests along streams the three species may coexist. *Colletia hystrix* grows in humid forests, and may also occur in the lakeshores of humid regions.

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The occurrence of *D. trinervis*-infective *Frankia* propagules was found in all soils from a vegetation gradient, with or without actinorhizal plants, from rainforest to the steppe in this region. Soils belonging to drier environments along water courses or with temporary flooding had higher nodulation capacities than other tested soils from the gradient, ranging from 25 to 340 infective units g^{-1} soil (Chaia et al., 2003).

This study was performed in order to know whether Andean lake sediments could have *Frankia* propagules infective in *Discaria trinervis*. The age of the sediments allows to determine the retention time of the infectivity of *Frankia* propagules.

2. Materials and Methods

Study area

The study area is located in the Nahuel Huapi National Park (41°36'S, 71°77'W and 40°08'S, 71°2'W), in the Andean Patagonia (Argentina). The climate is temperate cool, with a steep precipitation gradient in only 50–100 km eastward, from 3000 to 700 mm yr^{-1}, and predominant winds running from West to East (Barros et al., 1983). Soils, derived mainly from volcanic ash, are poorly developed, though rich in allophane, a poor defined aluminosilicate mineral (Mazzarino et al., 1998; Brady and Weil, 2002). The vegetation varies along the gradient from a dense rainforest (dominated by *Nothofagus dombeyi* and *Fitzroya cupressoides*) in the West, to semi-arid steppe in the East (Roig, 1998), where the rainforest area is a more pristine area of the national park.

The sampled lakes, namely El Trébol, Nahuel Huapi, Traful, and Espejo Chico, are a part of an important hydrographic system that includes many glacial lakes and low order streams and rivers (Modenutti et al., 1998a) (Fig. 1). The thermal regime of most lakes is almost warm monomictic with a period of summer stratification and the thermocline is frequently very deep, reaching 30–40 m (Modenutti et al., 1998b). Lake El Trébol tends to be dimictic as it freezes during hard winters; otherwise, its behaviour is also warm monomictic (Balseiro et al., 1997). The trophic level of these lakes is oligotrophic to ultra-oligotrophic (Modenutti et al., 1998b).

Sample collection, conditioning and dating

A sediment core (up to ca. 70 cm long) was extracted in each lake sampling site with a gravity-activated messenger corer, which included a disposable hollow cylinder to collect the sample. Unperturbed sediment cores were cut open, visually inspected, and sectioned at intervals of about every ca. 1 or 2 cm with an acrylic device. Each core section was denominated by a core code, according to the lake being sampled (TRE: Lake El Trébol, BL and BRC: Lake Nahuel Huapi, TRA: Lake Traful, and ECH: Lake Espejo Chico) and to the depth in the sediment core (e.g. 0–1, the upper slice excised in the core from 0 to 1 cm depth).

Each slice was lyophilized and homogenized in a plastic vessel with an agate hand. All tools and trays were thoroughly rinsed in 96% (v/v) ethanol before use. Part of the sediment samples was used to analyse heavy metal contamination. The sediments were dated by the ^{210}Pb and ^{137}Cs techniques to identify the historical changes in the lake's environment. ^{210}Pb and ^{137}Cs specific activity profiles were determined by high-resolution gamma ray spectrometry for core dating. The efficiency calibration for the peaks of interest was performed by using three different standards: the Standard Reference Materials IAEA-300 Baltic Sea Sediments, NIST Fresh Water Lake Sediment and NIST Peruvian Soil. The Constant Rate of Supply model was used for ^{210}Pb dating. For ^{137}Cs dating, specific activity profiles were compared with fallout sequence determined in this region, associated mainly with South Pacific nuclear tests from 1966 to 1974. Sediment samples were analyzed by Instrumental Neutron Activation Analysis. The samples were irradiated in the RA-6 nuclear reactor, Centro Atómico Bariloche. Sample masses of core sediments were around 150 mg. Standard reference materials were also analyzed to check the quality of the analyses. The results of the analyses showed good agreement with certified values. The consistency of results obtained with the studies on elemental analysis and dating showed that sediments were not mixed after sampling (Ribeiro Guevara and Arribére, 2002; Ribeiro Guevara et al., 2003b,c, 2005).

Moreover, three soil samples (ca. 0.5 kg each one) were randomly collected at 0 to 15 cm depth, under Rhamnaceae plants if present or under the dominant plant species, by the coast where each sediment core was obtained. Two additional soil samples were collected: one under a dense *D. chacaye* scrub in Puerto Blest (Blest), which is located in the rainforest area of the Nahuel Huapi lakeshore, and the other under naturally growing *D. trinervis* plants in Virgen de las Nieves (VIR), near Lake El Trébol (Fig. 1).

The soil samples taken from the same site were combined and air-dried. A subsample of soils was lyophilized to determine if the infectious capacities could be affected by the conservation procedure. Also, a characterization of the vegetation in the lakeshore close to the cores being sampled was performed. A description of sites and sampling data of sediment cores is included in Table 1.

Plant material and growth conditions

We used *D. trinervis* as a trap plant because soils from the studied area are infective in this plant species (Chaia et al., 2003). Moreover, strains isolated from *D. articulata*, *D. chacaye*, and *D. trinervis*, are compatible and effective in cross-inoculation assays within the genus *Discaria* (Wall et al., 2000). To test soil and sediment infectivity,

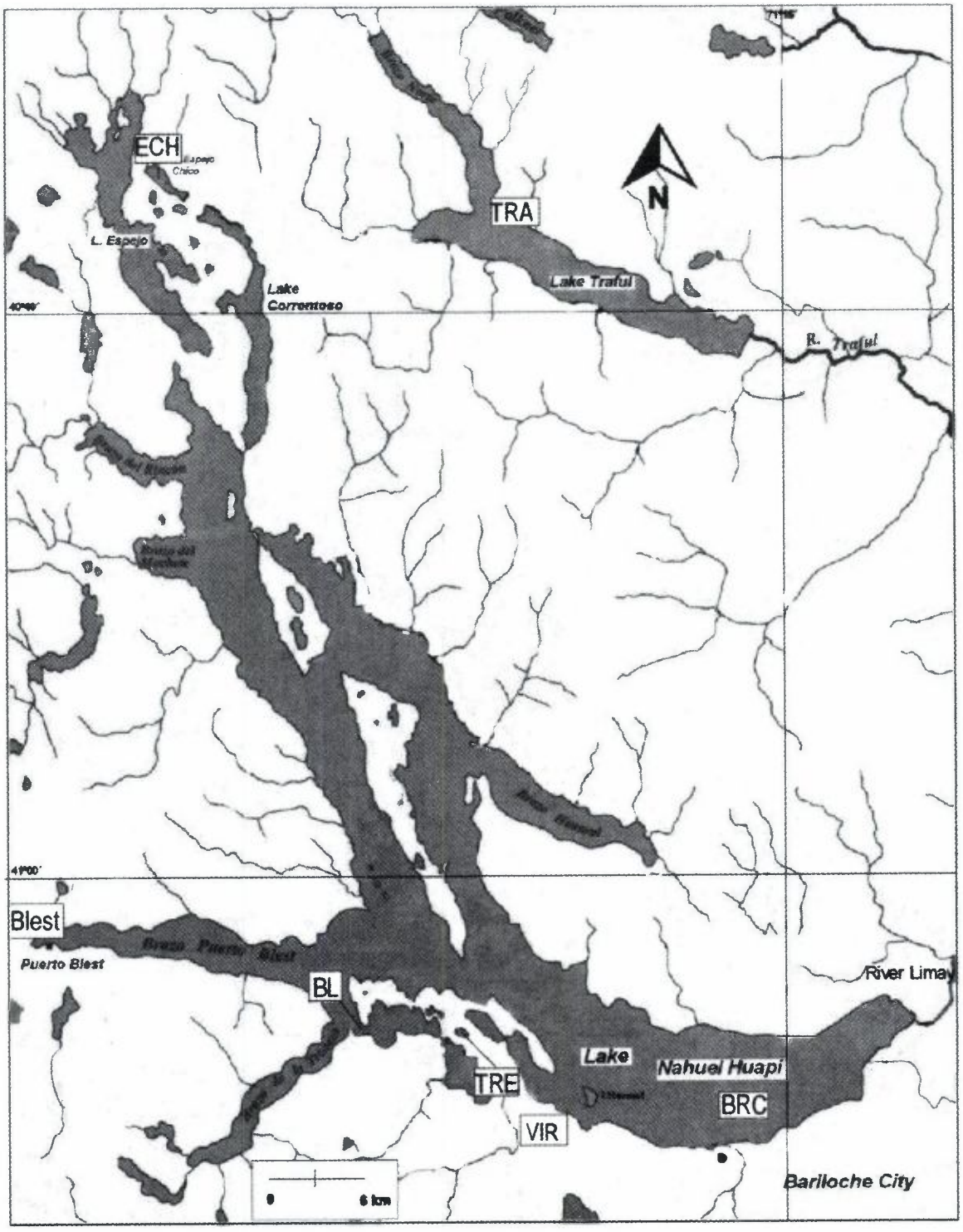

Figure 1. Sampling sites in glacial Andean lakes in northwestern Patagonia. Names inside boxes are the codes for the cores sampled in different lakes, namely: TRE, lake El Trébol; BL and BRC, lake Nahuel Huapi; TRA, lake Traful; and ECH, lake Espejo Chico. VIR (Virgen de las Nieves) and Blest (Puerto Blest) are soils samples collected under *Discaria trinervis* and *D. chacaye* plants, respectively.

D. trinervis seeds (origin S.C. de Bariloche), were scarified and germinated in sterile vermiculite moistened with Evans solution diluted to 1/10 strength supplemented with 0.71 mM N as NH_4NO_3 (Huss-Danell, 1978).

Germination and further plant growth, during 14 weeks after inoculation, were carried out in a growth chamber with 16 hs photoperiod provided by metal halogen lamps (Philips HPI-T 400W and Philips SON-T Plus 400)

Table 1. Description of sites and sampling data of sediment cores.

Site lake, core code Actinorhizal plants and main species in lakeshores	Date	Distance to shore-line[a] (m)	Water depth[b] (m)	Lake surface[b] Temp. (°C)	pH	O_2 (mg l^{-1})	Sediment Density[b] (g cm^{-3})	Organic matter[b] (%)	Sedim. rate[c] (mg cm^{-2} yr^{-1})
El Trebol, TRE *Discaria chacaye*, *Colletia hystrix*, *Schoenoplectus californicus*, *Chusquea culeou*, *Nothofagus dombeyi*, *N. antarctica*, *Diostea juncea*	15 June 2000	20	10	6.0	8.1	11.1	0.04	26.5	22.0
Nahuel Huapi, BL *D. chacaye*, *C. hystrix*, *Sarothamnus scoparius*, *Austrocedrus chilensis*, *Aristotelia chilensis*, *N. dombeyi*	15 August 2001	100	18	7.6	8.3	nd[d]	0.51	5.5	48.3
Nahuel Huapi, BRC *D. chacaye*, *D. articulata*, *S. scoparius*, *Salix humboldtiana*, *Gunnera* sp., *Mulinum spinossum*, *Cortaderia araucana*	20 August 1998	150	42	nd	nd	nd	1.19	3.1	59.0
Traful, TRA *D. chacaye*, *Aristotelia chilensis*, *N. dombeyi*, *Luma apiculata*, *Diostea juncea*, *Austrocedrus chilensis*, *Gunnera* sp.	27 Sept. 2001	400	18	8.9	7.9	11.5	0.33	16.8	41.7
Espejo Chico, ECH *D. chacaye*, *C. culeou*, *N. dombeyi*, *Austrocedrus chilensis*, *Maytenus chubutensis*, *Gunnera* sp., *N. antarctica*	25 October 2001	150	28	10.9	7.7	11.2	0.24	11.7	nd

[a]Estimated distance; [b]Data belong to the top layer of sediment cores (Ribeiro Guevara et al, 2003a), [c]Sedim. rate: sedimentation rate (Ribeiro Guevara et al., 2003b); [d]nd: not determined.

(photosynthetically active radiation was ca. 320 µmol m^{-2} s^{-1}). Average minimum and maximum temperatures were 21 and 26°C, respectively, while average relative humidity was 24%.

Plant growth and inoculation with sediments

To test 24 sediment slices from the TRE core for *Frankia* infectivity, two seedlings at the cotyledonary stage were each transferred to a sterile glass tube containing 30 ml of a mixture of sterilised sand and vermiculite (1:1 v/v) and ca. 20–25 mg of each slice. Positive controls were inoculated with the sediment sample and *Frankia* strain BCU110501 (Chaia, 1998). Negative controls were non-inoculated. Seedlings were weekly fertilized with 1/10 Evans solution with N 0.071 mM. After 30 days the plants were fertilized with the same solution without N.

To test infectivity of 4 sediment slices from BL, 6 slices from BRC, 4 slices from TRA, and 4 slices from ECH cores, three seedlings at the cotyledonary stage were aseptically transferred to growth pouches (Mega International, Minneapolis, USA) moistened with 1/10 Evans solution with N 0.71 mM. After 30 days the seedlings were inoculated with ca. 40–50 mg of each slice and the N of the nutrient solution was reduced to 0.071 mM. Negative controls were non-inoculated. Positive controls were inoculated as in the former assay. About 3 weeks after inoculation the bottom of each pouch was cut

off and all pouches belonging to the same treatment were placed together into a separate larger plastic bag. Nutrient solution was added to a level of about 1 cm and was refilled as necessary or renewed at least once a week (Wall and Huss-Danell, 1997). When nodules were clearly visible to the naked eye, the watering solution was replaced with 1/10 Evans without N.

Plant growth and inoculation with dried and lyophilized soils

The change in infectious capacity of lyophilized samples was determined by comparing plants inoculated with air-dried soils from lakeshores with plants inoculated with a subsample of the respective lyophilized soil. Two assays were performed with the same inocula doses and experimental conditions used for the sediments. The TRE coastal soil dried and lyophilized (TRE dried and lyoph) was tested in test tubes. Dried and lyophilized soil samples from BL, BRC, TRA, and ECH, were tested in pouches. Considering that the infective capacity of BL and BRC coastal soils could be affected by the human settlement of the city of San Carlos de Bariloche, the Blest sample, using again the pouch system, was also included in the these assays.

The inocula doses used in the previous assays (20–50 mg) is within the detection limit range found for *Frankia* propagules in soils of two sites in the Nahuel Huapi lakeshores. The lower infective dose of inocula which nodulated *D. trinervis* plants was ca. 10 mg. The studied sites had about 60 *Frankia* infective units per gram of soil (Chaia et al., 2003).

An additional infectivity assay was performed with a higher dose of coastal dried and lyophilized soils inocula. Two *D. trinervis* seedlings were grown in tubes filled with sterilized sand and vermiculite mixed with ca. 10 g of each soil sample. The plants were watered when necessary.

The VIR air-dried or lyophilized sample was tested by the same procedure than TRE coastal soil to determine if a soil under *D. trinervis* could have a different infective capacity than soils under *D. chacaye*, which was the main actinorhizal plant in lakeshores.

Measurements and statistical analysis

The appearance of nodulation in plants grown in pouches was recorded at weekly intervals after inoculation. The shoot height, root length, number of shoot internodes, dry weight of shoots and roots, and the number of nodules per plant, for plants inoculated at low dose of inocula, were recorded at the end of the experiments. Nodule lobes of some nodules were excised in slices by hand and were examined under an Olympus light microscope to determine the presence of *Frankia* vesicles.

Treatments were run with six replicates. VIR samples and positive controls were tested with three replicates. Data were calculated as average per plant within each pouch or tube.

Growth parameters of plants inoculated with sediments were compared with negative controls by using Kruskal-Wallis and Dunn's tests. Growth parameters of plants inoculated with air-dried and lyophilized coastal soils were compared by using Mann-Whitney U-test ($P<0.05$) (Zar, 1999).

3. Results

Occurrence of D. trinervis-*infective* Frankia *in sediments*

Table 2 summarizes the nodulation observed on test plants. The superficial top layer of each sediment core corresponding to four Andean lakes was *Frankia*-infective in *D. trinervis* plants. Moreover, samples corresponding to 1–2 cm depth from BL and BRC cores, or 1.5–2.5 cm depth from TRA core caused nodulation. The nodulation could be observed after 4 to 8 weeks of inoculation time. Infective samples had an estimated age up to 47 yrs.

The subsequent deeper slices of the sediment cores were not infective: 23 slices from the TRE core (TRE 2–4 to TRE 27–28) with an estimated age of up to ca. 180 yrs; BL 2–3 and BL 3–4, with an accumulation period of 12 yrs each and an estimated age of 25 to 49 yrs; 4 slices from the BRC core (BRC 2–4, BRC 4–6, BRC 10–12 and BRC 16–18), of ca. 47 to 420 yrs; TRA 0–5 and TRA 2.5–3.5 (of ca. 21 to 30 yrs); ECH 0.7–2, ECH 2–3 and ECH 3–4 (not dated due to the high interference of volcanic ashes in upper layers). 100% of TRE positive control plants were nodulated. The respective positive controls in pouch-grown seedlings started nodulation after two to nine weeks of being inoculated with the *Frankia* strain, as follows: all control plants from the two BL samples and from BRC 2–4, one control plant from each TRA sample, all control plants from ECH 0.7–2, and two control plants from the older ECH sediment layers.

Infective samples gave mean values between 1 and 7 nodules per plant. All nodulated plants had a green foliage and vesicles inside the nodules, but only the superficial top sediment layer from BRC core caused a higher shoot height and number of internodes than the negative controls ($P<0.05$) (See Table 3). The low number of nodules per plant may be thought as contamination; however, negative controls were never nodulated and had pale leaves. The high nodulation frequency in the positive controls (see Table 2) indicates that growth conditions during the experiments permitted nodulation to occur in all sediments or soils tested (Huss-Danell et al., 1999).

Frankia *infectivity of lyophilized soils*

Air-dried and lyophilized soils from lakeshores near TRE, BRC, and TRA cores, inoculated at low (Table 2) and at

Table 2. Occurrence of nodules on *Discaria trinervis* seedlings inoculated with ca. 20–50 mg of lyophilized sediments and coastal air-dried (dried) or lyophilized (lyoph) soils from Andean lakes. Inoculum codes are described in Table 1. The numbers beside each sample indicate the depth (cm) in the sediment core. VIR (Virgen de las Nieves) and Blest (Puerto Blest) are soil samples collected under *D. trinervis* and *D. chacaye* plants, respectively.

Inoculum	Period[a] (yr)	Growth system[b]	Test plant nodulation[c]			Positive sample control nodulation[d]		
			Time (wk)	Replicate	Plant	Time (wk)	Replicate	Plant
Sediments								
TRE 0–2	0–2.1	T	nd[e]	2/6	2/6	nd	3/3	12/12
BL 0–1	0–12	GP	7	2/6	4/18	4	3/3	4/4
BL 1–2	12–25	GP	7	4/6	9/18	2	3/3	6/6
BRC 0–1	0–23	GP	4	5/5	9/15	–	–	–
BRC 1–2	23–47	GP	5	2/3	2/7	9	3/3	4/6
TRA 0–0.5	0–4.3	GP	8	3/6	5/16	8	1/1	1/1
TRA 0.5–1.5	4.3–13	GP	–	0/6	0/16	4	1/1	1/1
TRA 1.5–2.5	13–21	GP	8	2/6	2/18	2	2/2	5/5
ECH 0–0.7	va[f]	GP	4	1/6	1/14	2	3/3	4/4
Coastal soils								
TRE lyoph	na[g]	T	nd	6/6	11/11	–	–	–
TRE dried	na	T	nd	6/6	10/10	nd	3/3	6/6
BL lyoph	na	GP	–	0/6	0/8	3	3/3	5/6
BL dried	na	GP	–	0/6	0/13	2	3/3	5/5
BRC lyoph	na	GP	4	6/6	11/13	4	3/3	6/6
BRC dried	na	GP	3	6/6	12/12	3	3/3	6/6
TRA lyoph	na	GP	4	1/3	1/4	2	3/3	9/9
TRA dried	na	GP	3	4/4	5/7	2	3/3	7/7
ECH lyoph	na	GP	–	0/6	0/13	4	3/3	6/6
ECH dried	na	GP	–	0/6	0/13	2	3/3	6/6
Blest lyoph	na	GP	4	2/2	2/6	–	–	–
Blest dried	na	GP	3	4/5	9/13	–	–	–
VIR lyoph	na	T	nd	3/3	5/5	–	–	–
VIR dried	na	T	nd	3/3	6/6	–	–	–

[a]Period: Sediment accumulation period (deep layers accumulation periods are obtained from extrapolation of core dating in upper layers) (Ribeiro Guevara et al., 2003b); [b]Growth systems are abbreviated: GP: growth pouch, T: test tubes with sand and vermiculite; [c]Test plant nodulation: Time refers to appearance of nodulation following inoculation, Replicate refers to number of replicated tubes or pouches with nodulated plants/total number of replicates, Plant refers to number of nodulated seedlings/number of seedlings tested; [d]Positive sample controls: plants inoculated with the soil or sediment sample and *Frankia* BCU110501; [e]nd: not determined; [f]va: sediment sequences of ECH were not dated by the ^{210}Pb or ^{137}Cs techniques due to the high interference of volcanic ashes in upper layers; [g]na: not applicable, only sediments were dated.

high doses (data not shown), were *Frankia*-infective in *D. trinervis* plants. ECH coastal soil caused nodulation only when was inoculated with a high dose. The BL coastal soil was not infective at any dose of inocula, but the inoculated plants had green foliage, probably due to high nitrogen content in soil. Blest coastal soil from Nahuel Huapi lakeshore caused nodulation in *D. trinervis* plants.

Nodulated plants had a green foliage and between 3 and 7 nodules per plant with vesicles inside. Only the coastal lyophilized Trébol soil had a higher shoot height and number of internodes than the negative controls ($P<0.05$) (Table 3). Lyophilization did not affect the infectivity of the coastal soil samples as corroborated by similar nodule numbers and growth parameters in plants inoculated with air-dried or freeze-dried soils ($P<0.05$).

Plants inoculated with soil collected under *D. trinervis* plants (VIR) had a similar number of nodules per plant and growth parameters than plants inoculated with coastal TRE soil ($P>0.05$) (Table 3).

4. Discussion

The presence of *D. trinervis*-infective *Frankia* in the proximity to watercourses, like in the Nahuel Huapi lakeshore, and in the riverbank of its effluent, River Limay, in NW Patagonia, was previously observed through infectivity tests (Chaia et al., 2003). The sites in the study area had actinorhizal nodulated plants in the shores (Chaia, 1997).

Therefore, we anticipated that *Frankia*-infective propagules could be also present in the Andean lake sediments.

In this study, the occurrence of *Frankia*-infective in *D. trinervis* from lyophilized sediments was determined for individual cores. Bowman and McCuaig (2003) also used a core to study prokaryotic diversity in a continental shelf. Although such an approach disregards the local variability, it allowed us to have a qualitative estimate of the occurrence of *Frankia* propagules in the Andean glacial lake sediments.

Table 3. Mean growth (and standard deviation) of *Discaria trinervis* seedlings inoculated and nodulated with lyophilized sediments and with coastal air-dried (dried) or lyophilized (lyoph) soils from Andean lakes. Sample codes and growth systems are described in Tables 1 and 2.

Inoculum	Growth system	N	Shoot height (cm)	Root length (cm)	Shoot internodes (number)	Nodules per plant (number)	Shoot DW (mg)	Root DW (mg)
Sediments								
TRE F 0–2	T	2	4.5 (–)	14.0 (–)	6.0 (–)	4.5 (–)	nd[a]	nd
BL 0–1	GP	2	1.9 (–)	12.5 (–)	4.0 (–)	5.8 (–)	4.5 (–)	7.6 (–)
BL 1–2	GP	4	1.4 (0.5)	14.3 (2.2)	3.1 (0.6)	7.1 (2.8)	3.3 (1.6)	7.6 (3.7)
BRC 0–1	GP	5	5.8 (1.8)*	14.4 (1.1)	6.2 (2.0)*	1.6 (0.4)	nd	nd
BRC 1–2	GP	2	1.6 (–)	14.5 (–)	3.8 (–)	5.0 (–)	2.8 (–)	5.1 (–)
TRA 0–0.5	GP	3	1.8 (0.7)	16.5 (2.1)	4.0 (1.0)	1.8 (1.4)	4.1 (2.6)	7.8 (1.9)
TRA 1.5–2.5	GP	2	1.5 (–)	15.5 (–)	3.0 (–)	1.5 (–)	2.5 (–)	5.7 (–)
ECH 0–0.7	GP	1	1.0 (–)	15.0 (–)	2.0 (–)	1.0 (–)	1.4 (–)	6.3 (–)
Coastal soils								
TRE lyoph	T	6	4.6 (1.4)*	13.8 (2.1)	8.9 (2.9)*	5.0 (2.0)	nd	nd
TRE dried	T	6	6.2 (3.4)	15.5 (2.4)	9.1 (2.5)	5.7 (1.7)	nd	nd
BRC lyoph	GP	6	2.0 (0.5)	13.3 (1.8)	4.3 (0.5)	6.2 (2.0)	3.1 (0.9)	7.2 (0.5)
BRC dried	GP	6	2.0 (1.0)	14.6 (2.2)	4.4 (1.8)	5.5 (2.4)	6.4 (6.7)	14.9 (11.6)
TRA lyoph	GP	1	1.0 (–)	13.0 (–)	4.0 (–)	5.0 (–)	4.0 (–)	4.5 (–)
TRA dried	GP	4	1.4 (0.5)	12.5 (1.3)	4.0 (0.8)	5.6 (3.6)	3.3 (1.1)	7.3 (2.8)
Blest dried	GP	4	5.0 (0.7)	12.5 (0.0)	5.4 (1.6)	2.8 (0.5)	nd	nd
Blest lyoph	GP	2	3.5 (–)	12.5 (–)	4.0 (–)	3.0 (–)	nd	nd
VIR lyoph	T	3	6.0 (1.0)*	14.5 (0.9)	9.5 (1.3)*	7.3 (3.4)	nd	nd
VIR dried	T	3	4.5 (0.5)	13.3 (1.2)	7.3 (1.2)	6.7 (4.5)	nd	nd

[a]nd: not determined. *Significant differences with the negative control corresponding to the same growth system (Kruskal Wallis and Dunn's Tests).

Different storage conditions may reduce *Frankia* infectivity for the host plant species (Maunuksela et al., 2000; Sayed et al., 1997). Although we did not test the infectious capacities of fresh soils, we corroborated that nodulation capacity was similar for lyophilized and air-dried soils (Table 2).

We found that all superficial sediment layers from the four sampled lakes caused nodulation in *D. trinervis* but only subsurface sediments of lakes Nahuel Huapi and Traful were infective (Table 2). Although all infected plants with sediments had green foliage and vesicles inside the nodules, only the plants inoculated with the superficial slice of BRC core had a higher growth than the negative controls, seeming to have an effective symbiosis. The small inoculum dose used could be a reason for the low nodulation and an apparent low effectiveness (suggested by the green foliage in contrast to the pale leaves of negative controls) in most nodulated plants during the experiment. Perhaps, a lower compatibility of the *Frankia* sediment or soil populations with the trap plant, *D. trinervis*, could also account for this result, since most actinorhizal plants occurring in the study area belonged to *D. chacaye* species. The higher growth of plants inoculated with soils collected under *D. trinervis* plants (VIR), when compared to the negative controls, could support this assumption (Table 3).

It is possible that the occurrence of *Frankia* propagules in sediments, at distances of about 20 to 400 m from shorelines, and at water depths of 10 to 42 m, could be due to water transport, although we cannot discard wind dispersal (Wijnholds and Young, 2000). Hence, *Frankia* propagules in upper layers of the sediments, could possibly be moved by water and eventually be a source of infection for potential host plants in the shores (Huss-Danell et al., 1997). This assumption would be supported by the fact that water movements during the mixing periods favour the removal of particles in superficial sediments of the glacial Andean lakes (B. Modenutti, personal communication). Therefore, although *Frankia* was not detected in lake Michigan waters (Batzli et al., 2004), we suggest that *Frankia* propagules would be also be present in the water column, in agreement with the finding of *Alnus*-infective *Frankia* in river waters (Huss-Danell et al., 1997).

The estimated age of subsurface sediments containing *Frankia*-infective was up to ca. 50 yrs. This value reveals the retention time of *Frankia* propagules infectiveness. This infectivity retention time is in the range of that found by Huss-Danell et al. (1997) for *Frankia* infective-*Alnus* in varved sediments of a lake near the Baltic Sea. Considering that several *Frankia* strains isolated from *D. articulata*, *D. chacaye* and *D. trinervis* are capable of producing spores in culture (Chaia, 1998, 1997), it is conceivable that in our study the infective propagules were spores.

It would be difficult to make further considerations about *Frankia* survival in sediments and its infectious capacity as

related to the presence of chemical compounds or physicochemical characteristics of the sediments, considering the qualitative character of the assays. Anyhow, we would like to briefly comment that the upper layers of the sediment cores had some heavy metals contents that were above base line values (Ribeiro Guevara et al., 2003c), particularly silver in BRC and BL cores (Ribeiro Guevara et al., 2005). Nevertheless, these values were appreciably lower than those reported to be inhibitory (<0.5 mM) for the growth of several *Frankia* strains (Richards et al., 2002).

In conclusion, our results support previous studies (Huss-Danell et al., 1997), which found *Frankia*-infective propagules in lake sediments. We suggest that, in addition to other transporting agents already reported, namely, river waters (Huss-Danell et al., 1997), wind (Zitzer and Dawson, 1992), and animals (Paschke and Dawson, 1993; Redell and Spain, 1991), *Frankia* propagules are also transported by the Andean lake waters, thus allowing *Frankia* dispersal to new environments.

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