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Evaluation of the genotype-environment interaction in the establishment of *Lotus uliginosus* (Schkuhr) with soil-cores

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Introduction

Lotus uliginosus Schkuhr (big trefoil) is a perennial legume highly adapted to different Uruguayan soils. Its extensive underground system formed by the crown and central taproot gives origin to a network of rhizomes, stolons and fibrous roots that successfully colonizes the native swards (Carámbula *et al.*, 1994). The tetraploid cultivar Grasslands Maku (Maku) is the most utilized in the country, even when other diploid cultivars have been evaluated (Castaño and Menéndez, 1998). Maku, released in 1975 in New Zealand, was bred from local ecotypes and introductions from Portugal with outstanding winter growth (Charlton, 1983). Chromosome duplication increased its seed size and seedling vigour. The persistence and high forage production are the main characteristics for the improvement of natural grasslands in extensive cattle raising areas, particularly in the East of the country (Risso *et al.*, 1990; Carámbula *et al.*, 1994; Carámbula *et al.*, 1996; Castaño and Menéndez, 1998). In these conditions, Maku shows an improved performance compared to other legumes of well-known productive capacity, such as *Lotus corniculatus* (Risso and Berretta, 1996; Castaño and Menéndez, 1998).

The vegetative growth of Maku is opposed to the low seed production under Uruguayan environmental conditions. For this reason INIA's breeding program was aimed to obtain an adapted big trefoil with high seed production. The experimental line LE 627 is an early type diploid material, with good initial growth and high seed potential. Regional evaluation of LE 627 produced inconsistent results; its slow establishment and short persistence has been verified in the basaltic soils, in opposition to the results in the Eastern region, where the experiments have been successfully established (Risso D. *Com. Pers;* Iglesias and Ramos, 2003; INASE, 2005; Castaño and Menéndez, 1998). This differential performance is probably due to a genotype-environment interaction, either by a specific adaptation of this genotype to different soils, to environmental conditions or an interaction in the symbiotic relationship.

A clear example of symbiotic interactions happens with the introduction of species of the genus *Lotus* and rhizobia strains that can be effective or parasitic according with host

combination (Pérez and Labandera, 1998; Irrisarri *et al.*, 1996). Rhizobia strains able to produce nodules in *Lotus* spp. belong to *Rhizobium loti* and *Bradyrhizobium* sp., with a relative specificity between the species and its symbionts. *L. corniculatus* and *L. glaber* form a symbiotic effective group with fast growing strains (*R. loti*) whereas *Lotus subbiflorus* and *L. uliginosus* form another effective group with slow growing strains (*Bradyrhizobium* sp.; Brockwell *et al*., 1994; Baraibar *et al.*, 1999). These symbiotic groups have incompatible relationships to each other: the bacteria of one symbiotic group produces nodules in the other group host but the relationships are ineffective or parasitic. Under these conditions there is no nitrogen fixation because functional symbiosis does not occur, and so establishment difficulties might arise. Lieven-Antoniou and Whittam (1997) reported mechanisms that lead to differential recognition of host genotypes (*L. corniculatus*) and their symbionts (*R. loti*). However, there are fast growing strains (NZP2037) that form effective nodules in both symbiotic groups, although their effectiveness is not as high as the specific ones (Labandera C. *Com.Pers.*; Pankhurst, 1981; Scott *et al.*, 1987; Barrientos *et al*, 2002). The rhizobia strain selection programs for *L. subbiflorus* and *L. uliginosus* has showed a much better efficiency with slow growing srains (Mayans 2003). The rhizobia strain collection of the Department of Soil Microbiology (RENARE-MGAP, Uruguay) holds several strains and isolations for species of the genus *Lotus*. Strain U526 (NZP 2309, New Zealand) is recommended for *L. uliginosus* in Uruguay, whereas strain 531 (NC3, Uruguay) is recommended for *L. subbiflorus*, although both strains are effective in either host (http://fp.chasque.apc.org:8081/microlab/LMSCI/catalogo/marco.htm).

Native or naturalized rhizobia that effectively nodulate the four *Lotus* species of agronomic value are generally present in Uruguayan soils. *L. corniculatus* has been utilized since the 60's in rotations with crops in the arable area. Therefore, these soils have an important concentration of effective strains for this host. However, without this previous history of cultivation, natural grasslands usually have native *Mesorhizobium loti*, with high variability in symbiotic efficiency (Baraibar *et al.*, 1999). Effective native rhizobia that nodulate *L. uliginosus* and *L. subbiflorus* are in very low concentrations and so, they do not compete with introduced strains. Nevertheless, with the introduction of inoculated legumes of the same genus, the rhizobia remain in the soil in important concentrations. If the *Lotus* species to be introduced are not of the same symbiotic group of the predominant rhizobia population in the soil, then defective establishment or yield losses might happen (Gwynne *et al.*, 1980). The establishment difficulties in these cases can be attributed to host-strain interactions with less efficiency in nitrogen fixation, which might restrict the symbiotic potential of the host. In conclusion, the successful establishment of species of the genus *Lotus* depends on the effectiveness of the rhizobia populations present in the soil and the appropriate management of the inoculation technique (Skerman *et al.*, 1991; Labandera *et al.*, 2005).

LE 627 has had a variable performance in the regional trials, with poor establishment in basaltic soils, generating the question about the role of soil types and the native rhizobia population on the establishment of this genotype. The objective of this research was to analyze host-rhizobia-soil interaction in the establishment of the symbiosis and initial development of *L. uliginosus*, using soil-cores under standardized environmental conditions.

Materials and Methods

The treatments consisted of 2 genotypes, with and without inoculation and 4 soil types in a complete factorial design with 6 replicates. Genotypes were cultivar G. Maku and the experimental line LE 627. Each genotype was sown with (WI) and without (NI) the rhizobia strain U-526. Inoculation was done by watering twice the recommended commercial dose.

The four soils are representative of the main areas of extensive production and have different physical-chemical characteristics (Table 1). These correspond to: (1) Eastern lowlands (Lowl) with rice stubble at Paso La Laguna (Treinta y Tres), representative of the lowest topographical levels in the Eastern plains. These humid, heavy soils have a shallow phreatic nape during most of the year. (2) Basaltic soil (Bas) with natural grassland at Glencoe (Paysandu), representative of medium soils of the basaltic region, where the landscape corresponds to hills and sharp valleys. These soils have frequently abundant thick fractions as gravels and stones and the dominant silt is montmorillonite. (3) Rolling hills (Rol) with natural grassland at Palo a Pique (Treinta y Tres), representative of the highest levels in the Eastern landscape known as "Lomadas del Este". The soil presents alternating higher and depressed areas formed by two profiles: superficial phase with horizon A thickness of 10 - 30 cm and deep phase where horizon A thickness is of 30 - 90 cm. (4) Medium granitic soil (Gra) with natural grassland at La Carolina (Flores), representative of the Central granitic region. This area has shallow soils associated with deep and more fertile soils developed from slime-loamy silts covering the granitic basement.

Table 1. Chemical characteristic of the soil modal profiles (Source: Ministerio de Agricultura y Pesca, 1979)

Location	Soil Unit	Predominant Soil	Horizon cm	pH (water) 1:2.5	$CEC_{pH\ 7}$*	$V(\%)_{pH\ 7}$
Lowl	La Charqueda	Solod Melanic	A_1, 0-17	5.5	13.0	52
Bas	Queguay Chico	Litosol Eutric Melanic	A_1, 0-12	5.9	42.6	79.1
Rol	José Pedro Várela	Argisol Subeutric Luvico	A_1, 0-21	5.5	18.5	55
Gra	La Carolina	Brunosol Eutric	A_1, 0-26	6.1	27.1	80.8

$CEC_{pH\ 7}$ = cationic exchange capacity at pH 7
$V(\%)_{pH\ 7}$ = (total base / $CEC_{pH\ 7}$)100

The soil samples were taken with a soil-corer of 8 cm diameter and 15 cm depth, maintaining intact the soil structure and the natural sward. The soil-cores were placed in plastic bags for watering and sown July 15th 2003 with approximately 20 viable seeds per soil-core. The

experiment was repeated in two contrasting environments in order to study the temperature effect on the variables under study during the establishment period. The experiments were located in greenhouses: one at 18-25ºC and another at 8-20ºC.

Vigour and initial development were monitored weekly. In September 2003, 10 weeks after sowing, a destructive evaluation of soil-cores was carried out by water immersion. The fresh aerial and root mass were separately weighed. Nodules size, quantity and localization were evaluated by visual estimation with an adapted scale of 10 values Master Class (2000; Table 2).

Tabla 2. Nodulation scale

Scale	Description	Scale	Description
1	Without effective nodules	**6**	Few effective nodules in main root and many nodules in secondary roots
2	Very few effective nodules in secondary roots	**7**	Few effective nodules only in main root
3	Few effective nodules in secondary roots	**8**	Crowns with few effective nodules
4	Many effective nodules in secondary roots	**9**	Crowns half-covered with effective nodules
5	Few effective nodules in main root and few nodulesI in secondary roots	**10**	Crowns completely covered with effective nodules

Results and discussion

The temperature had a great influence on big trefoil establishment, with greater growth and larger differences between treatments in the warm environment (18-25°C; Table 3 and 4). There was a triple interaction between genotypes, soils and inoculation at both temperatures on the aerial seedling growth (Table 3), indicating the differential symbiotic response of the genotypes in different soils. Maku responded to the inoculation with U-526 in Lowl in both temperatures (Figure 1a) and in Rol at 18-25°C (Figure 2a), while there was no effect of inoculation in Gra and Bas (Figures 3a and 4a) under both temperatures and in Rol at 8-20°C. In opposition, LE 627 only responded to the inoculation in Bas at 18-25°C (Figure 4b), although seedling aerial weight was smaller than the best growth obtained in Rol with or without inoculation (Figure 2b). Best seedling growth for both genotypes was always achieved in Rol, possibly indicating a favourable soil for *L. uliginosus* establishment and/or the presence of effective strains (Figure 5c). There was no response to rhizobia inoculation in Gra, probably as an indication of naturalized strains from *L. subbiflorus* history hindering the impact of inoculation.

Aerial mass data demonstrated the large influence of soil native or naturalized rhizobia populations in the seedlings development of both genotypes. In addition, they could indicate certain strain-host specificity, since only Maku responded to inoculation in Lowl, while LE 627 had a low response to inoculation in Bas not reaching the values obtained in Rol. These

results corroborated the good performance of the strain U-526 for Maku and the need for further strain selection under field conditions for the diploid LE 627, since genotype-strain interaction for diploid and tetraploid *L. uliginosus* has already been reported (Barrientos *et al.*, 2001).

Table 3. Fresh weight of aerial part (g/soil-core) for the different temperatures, genotypes, soils and inoculation treatments.

		Temperature			
		18-25ºC		8-20ºC	
***L. uliginosus* genotypes**		**LE 627**	**Maku**	**LE 627**	**Maku**
Soil	**Inoculation**				
Basaltic (Bas)	NI	0.54	1.17	0.47	0.79
	WI	1.61	1.32	0.30	0.47
Granitic (Gran)	NI	1.96	1.94	1.00	0.89
	WI	1.57	2.26	0.99	0.52
Rolling Hills (Rol)	NI	2.51	2.47	1.88	1.77
	WI	2.74	3.27	1.42	1.44
Lowlands (Lowl)	NI	1.34	1.09	1.00	0.52
	WI	1.74	3.35	1.07	2.59
LSD (5%)		0.78		0.71	
Significance		0.008		0.024	

Table 4. Root fresh weigh (g/soil-core) and nodulation scale for the different soils and inoculation treatments.

		Root weight		Nodutation Scale	
Temperatures		**18-25ºC**	**8-20ºC**	**18-25ºC**	**8-20ºC**
Soils	**Inoculation**				
Basaltic (Bas)	NI	0.25	0.18	3.29	3.38
	WI	0.34	0.15	8.21	6.10
Granitic (Gra)	NI	0.56	0.31	7.44	6.59
	WI	0.47	0.39	7.70	6.69
Rolling Hills (Rol)	NI	0.60	0.73	7.93	6.81
	WI	0.71	0.53	8.47	7.14
Lowlands (Lowl)	NI	0.34	0.24	5.56	5.65
	WI	0.62	0.39	9.06	8.72
LSD (5%)		0.15	0.15	1.17	1.50
Significance		0.015	0.01	<0.001	0.008

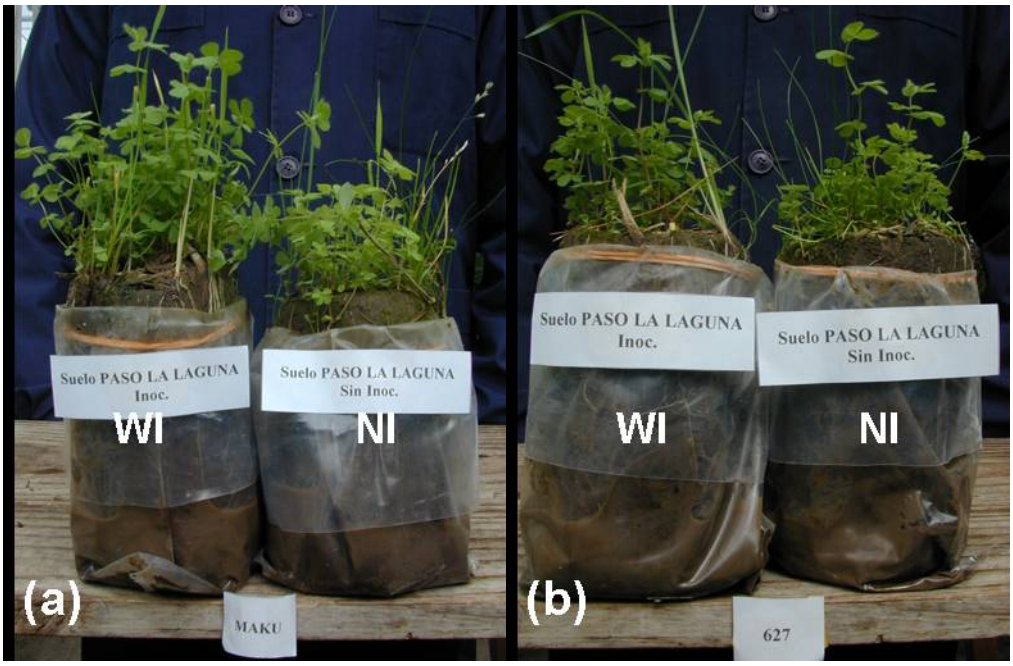

Figure 1. Lowland soil-cores (Lowl) grown at 18-25°C sown with Maku (a) and LE 627 (b). WI inoculated with U-526 and NI without inoculation.

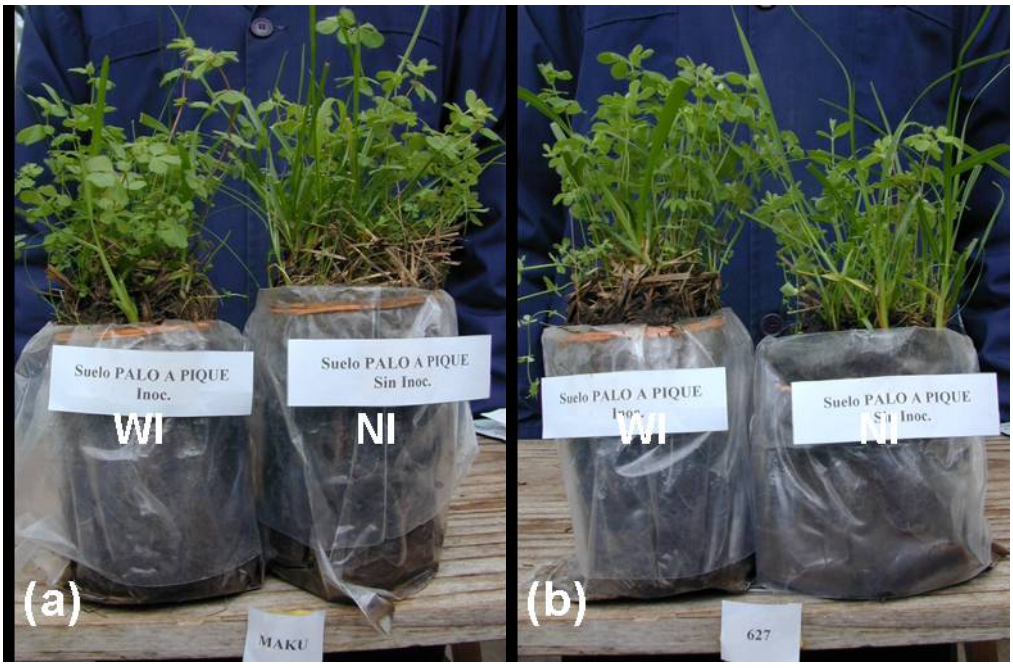

Figure 2. Rolling hills soil-cores (Rol) grown at 18-25°C sown with Maku (a) and LE 627 (b). WI inoculated with U-526 and NI without inoculation.

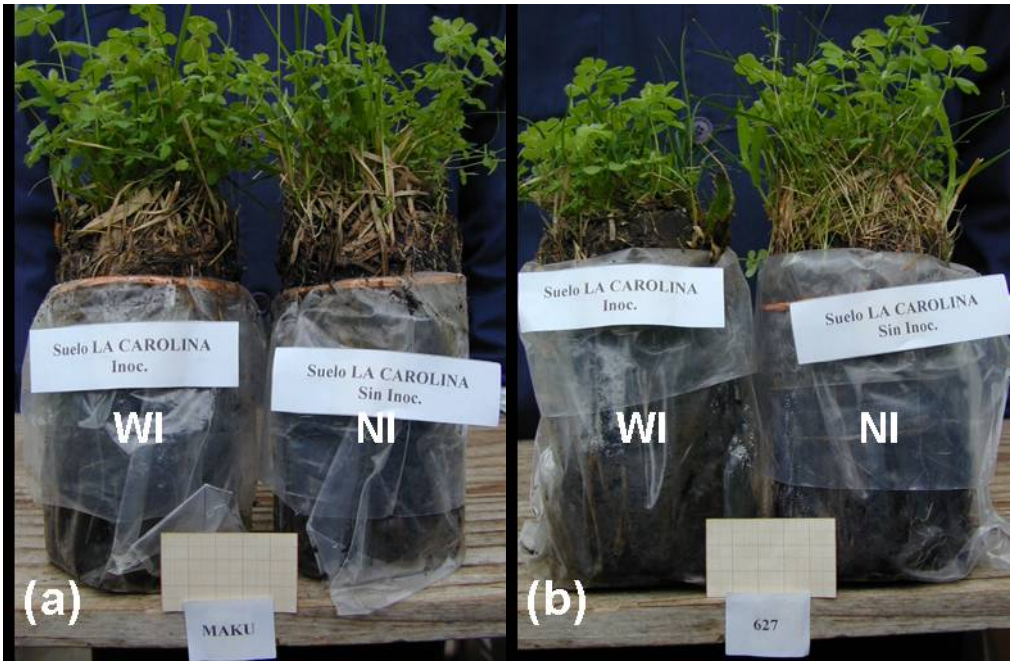

Figure 3. Granitic soil-cores (Gra) grown at 18-25°C sown with Maku (a) and LE 627 (b). WI inoculated with U-526 and NI without inoculation.

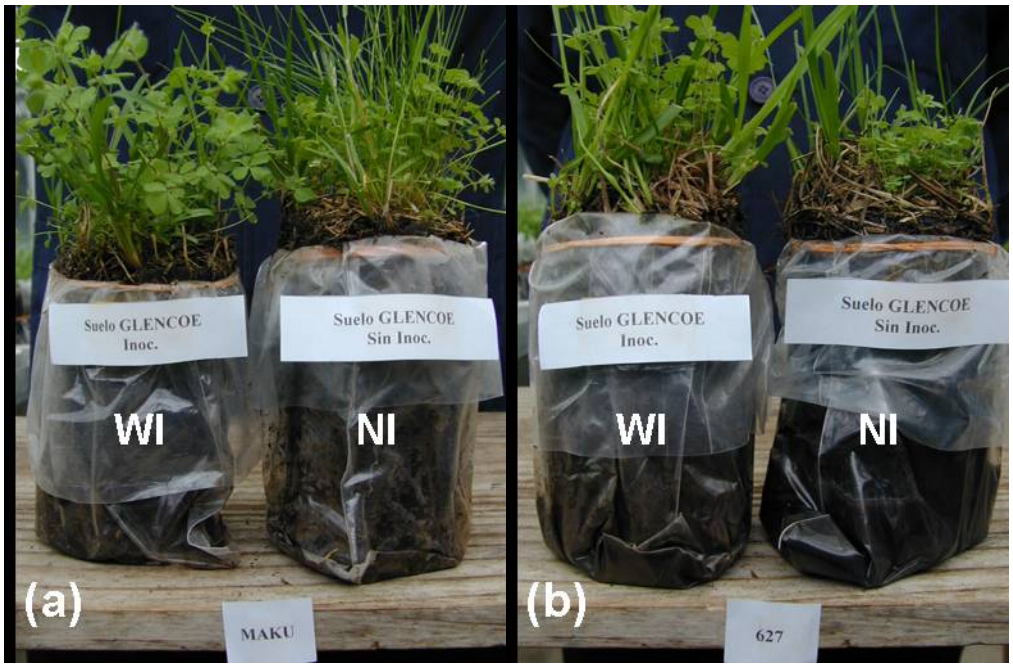

Figure 4. Basaltic soil-cores (Bas) grown at 18-25°C sown with Maku (a) and LE 627 (b). WI inoculated with U-526 and NI without inoculation.

Soil types had a great effect on big trefoil establishment (Figure 5). The soil main effect demonstrated that both genotypes had better performance at Rol than at Bas, in agreement with field observations (Risso, D. *Com. Pers;* Iglesias and Ramos, 2003; Carámbula *et al.*, 1996; Castaño and Menéndez, 1998), while the growth was intermediate in Lowl and Gra soils. Soil incidence in *L. uliginosus* growth was also observed in the root development (Table 4). The root growth was lower in Bas at 18-25°C in comparison with Rol and Gra, while there were only significant differences between Bas and Rol at 8-20°C. This data confirmed previous field experiment results (Iglesias and Ramos, 2003; Castaño and Menéndez, 1998), and discarded temperature as factor of the differential performance between big trefoil genotypes between regions.

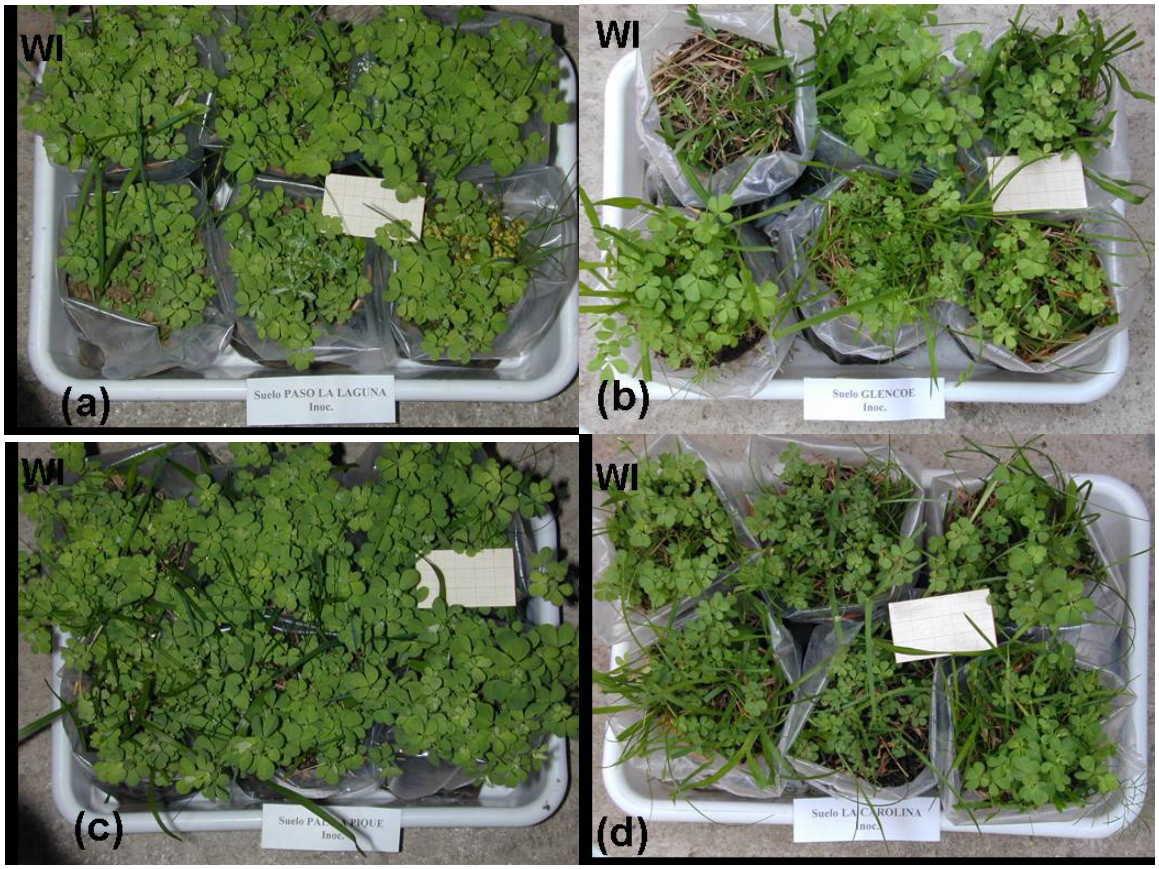

Figure 5. Inoculated soil cores grown at 18-25°C: (a) Eastern lowlands with rice stubble (Lowl); (b) basaltic soil with natural grassland (Bas); (3) rolling hills with natural grassland (Rol); (d) medium granitic soil with natural grassland (Gra).

Rhizobia inoculation had a strong interaction with soils on root development at both temperatures, since WI only had bigger roots in Lowl (Table 4). On the other hand, nodulation scale values in both Lowl and Bas were significantly higher in WI than in NI. Inoculation response for Bas was only recorded in the nodulation scale, since there was no significant ($P<0.05$) aerial or root growth improvement (Tables 3 and 4). These results could indicate the presence of other growth restrictions associated to the soils of the basaltic

region.

The high response to inoculation in Bas and Lowl could be explained by the absence or low concentration of effective rhizobia strains in these soils, confirming the impact of inoculation for these soils in the establishment and initial growth of *L. uliginosus* (Vance *et al*, 1987). In spite of field observations showing difficulties to achieve good and persistent stands of LE 627 in basaltic soils (D.Risso, *Com.Pers*.; Iglesias and Ramos, 2003), its seedling weight was similar ($P<0.05$) to Maku in this experiment, providing evidence for restrictions on establishment for both genotypes

The evaluation of soil-cores with natural grasslands allowed a first approach to the study of the complex of *L. uliginosus* establishment and its response to inoculation in different soils of Uruguay, by discarding the climatic differences among areas through the standardization of the environment. This research study has shown an interaction between host genotypes and U-526 inoculation related to the presence / absence of effective rhizobia strains in different soils. Similarly, difficulties of big trefoil establishment in basaltic soils are probably associated to soil physical characteristics. The understanding of factors involved in these genotype-soil interactions requires more precise research of longer duration, in order to recognize the soil characteristics that restrict establishment and to know the relationships between host and rhizobia genotypes, as well as native populations of rhizobia. Finally, it would be necessary to carry out more research and put greater effort in strain selection of specific rhizobia for the diploid genotype LE 627.

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