Acta Bot. Neerl. 29(5/6), November 1980, p. 429–441.

EVIDENCE FOR ECOTYPIC DIFFERENTIATION IN LUPINUS-ASSOCIATED RHIZOBIUM

D. M. PEGTEL

Botany Department, University of Washington, Seattle (USA)*

SUMMARY

In the vascular flora of the main rock-soil system known as serpentine south of Mt. Stuart of the Wenatchee Mountains of the Cascade Range (Washington, USA), leguminous species, such as members of the genus *Lupinus*, are rare.

Populations of *Lupinus laxiflorus*, *L. lepidus* var. *lobbii*, and *L. polyphyllus* were investigated. These species occur also on non-serpentine soils. The serpentine and non-serpentine populations therefore may be regarded as edaphic ecotypes. One of the main questions is whether the effect of serpentine soil is reflected in the physiology of N-fixing *Rhizobium lupini*.

The root systems of the three species are nodulated. There are no indications that serpentine populations have fewer root nodules than non-serpentine populations. Bacteroïds of *R. lupini* are present in all the nodules.

The strains of *R. lupini* all fix atmospheric nitrogen, but as compared to commercially available strains, their effectiveness is variable and in general low. Serpentine and non-serpentine strains do not differ materially. The available evidence indicates that strains of *R. lupini* differ physiologically, presumably in consonance with their edaphically differentiated host species, as far as nickel and magnesium are concerned.

It is therefore unlikely that the rarity of *Lupinus*-species on serpentine soil types is determined by the growth peculiarities of *R. lupini* under the prevailing habitat conditions.

1. INTRODUCTION

The best examples of soil effects on the distribution of plant species are from soils that develop on unusual formations. Among these are the uniquely unfavourable serpentine soils which occur in various areas all over the world.

The term "serpentine" soils encompasses ultramafic or serpentine rocks which are basically hydrated magnesium iron silicates of less than 40% silica, derived by metamorphosis from the predominantly olivine-containing igneous rocks dunite and peridotite, and the soils weathering therefrom (WALKER 1954, KRUCKEBERG 1967, PROCTOR & WOODELL 1975).

Wherever serpentines occur, they have distinctive plant communities and unique floras which sharply contrast with plant communities and floras of adjacent non-serpentine soils. The latter soils usually have a much more varied, luxurious vegetation. The plant life of serpentine areas varies greatly with location due to a wide range in the composition of the parent material, and consequently in the composition of the derived soils. It also varies with soil

* Present address: Vakgroep Plantenoecologie, Biologisch Centrum, Rijksuniversiteit Groningen, Postbus 14, 9750 AA Haren (Gn).

depth, topography, and physical factors (WALKER 1954, PROCTOR & WOODELL 1975).

The unique flora of serpentine habitats can be grouped into two categories: an assembly of species which are totally restricted to serpentine soils (narrow endemic species, MASON 1946), and are therefore the most reliable serpentine indicators (WHITTAKER 1960, KRUCKEBERG 1969); and an assembly of species which appear edaphically indifferent since they have populations on both serpentine and non-serpentine locations, and grow equally well on either type of soil (KRUCKEBERG 1951, 1954, 1964, 1967, 1969, WHITTAKER 1954, 1960, WARING 1969, PROCTOR & WOODELL 1975). KRUCKEBERG (1954, 1964, 1967, 1969) as well as WHITTAKER (1954, 1960) and WARING (1969) showed that in the process of evolution indifferent species have, by natural selection, evolved ecotypes each adapted to a specific habitat, either serpentine or non-serpentine.

The striking differences between serpentine floras and those of adjacent non-serpentine soils have led to attempts to explain the situation on the basis of edaphic factors. Soils derived from serpentine rock are generally low in the trace element molybdenum (WALKER 1948), and in major nutrients such as total and absorbed calcium, nitrate, phosphate, potassium, and sulfate. Magnesium, nickel and chromium are generally high (WALKER 1954, PROCTOR & WOODELL 1975).

Heavy metals such as nickel and chromium appear to determine, at least in part, the serpentine flora (BIRRELL & WRIGHT 1945, HUNTER & VERGNANO 1952, 1953, RUNE 1953, VERGNANO & HUNTER 1953, SOANE & SAUNDER 1959, SASSE 1979). However, WALKER (1954) and PROCTOR & WOODELL (1975) consider that there is insufficient evidence that nickel and chromium toxicity is as important as low calcium/magnesium levels.

Compiled values (PROCTOR & WOODELL 1975) indicate that exchangeable magnesium in temperate serpentine soils is relatively high, and ranges between 3 and 30 me/100 g of soil. KRUCKEBERG (1969) found quantities ranging between 0.5 and 34.7, and JONES et al. (1977) between 15.2 and 30.0. Exchangeable calcium is generally present in very small quantities in serpentine soil, ranging between 0.2 and 4 me/100 g of soil (PROCTOR & WOODELL 1975). Values of up to 12.4 are found by KRUCKEBERG (1969), and of up to 9.7 by JONES et al. (1977). It is obvious that calcium is generally in shorter supply than magnesium, and consequently the Ca/Mg ratio is less than 1, and usually below 0.4 (KRUCKEBERG 1967, 1969, PROCTOR & WOODELL 1975, SASSE 1979).

There is good reason to believe that the low calcium saturation of the serpentine soil is of major importance in the serpentine problem. Therefore, plants that grow well on such areas must, first of all, be tolerant to such a condition (WALKER 1954). It appears probable that serpentine endemics and serpentine populations of indifferent species can long persist in serpentine habitats because they possess the capacity to obtain calcium from soils of low calcium status. Plants lacking this ability will be excluded from them (KRUCKEBERG 1954, WALKER 1954, WHITTAKER 1954, MADHOK & WALKER 1969, MAIN 1974, PROCTOR & WOODELL 1975).

In the Western United States and specifically in the Wenatchee Mountains (Washington) atmospheric nitrogen fixing plant species are rare on serpentine. KRUCKEBERG (1969) found that *Lupinus laxiflorus* is the only representative of the family Leguminosae occurring on serpentine. This poses the question why leguminous species in general and *Lupinus* species in particular are found rarely on serpentine outcrops in that area.

WHITE (1967) observed that populations of the indifferent species *Ceanothus cuneatus* growing on and off serpentine soil types differ with respect to the percentage of nodulated individuals: 3 and 55%, respectively. He suggests that the paucity of nodulated plants on serpentine soils may be attributed especially to low molybdenum levels since N-fixing plant species require much more molybdenum than species which lack this ability. Although deficiences of molybdenum in the soil are general, there are differences between legume species in their response to fertilization with molybdenum (JOHNSON et al. 1952).

JONES et al. (1977) tested serpentine-derived soil from 23 sites in California to assay the fertility status for growth of inoculated *Trifolium subterraneum*. It was found that many of the soils are at least responsive to Ca and Mo. They did not report whether the plants were nodulated, but it is very likely that they were, since no nitrogen was supplied. So it seems that *Rhizobium* is also responsive to at least Ca supplied by the host plant. Mg can be left out of consideration since the Mg level is high.

The legume, *Pearsonia metallifera*, has 1.5% chromium and 10.3% nickel in the root ash when growing on a Rhodesian serpentine soil. At the same time the root systems were well nodulated with pink and active nodules (WILD 1974). Furthermore, individuals of the endemic species *Lotononis serpentinicola* and of the indifferent species *Sesbania microphylla* growing on the same serpentine soil were also well-nodulated. It seems therefore unlikely that nodulation and N-fixation are necessarily negatively affected by normally toxic concentrations of nickel and chromium (> 100 ppm, FINCK 1976), as suggested speculatively by WHITE (1967).

The purpose of our research was to determine whether the type of ecotypic differentiation in *Lupinus*-species on serpentine and non-serpentine soil types is reflected in the associated strains of *Rhizobium lupini*.

2. MATERIALS AND METHODS

2.1. Plant collections

Adult individuals of three perennial *Lupinus* species were randomly excavated from serpentine and non-serpentine localities in Washington State (USA) during the vegetation period of 1977. Populations of serpentine origin came from peridotite and/or serpentine outcrops in the Wenatchee Mountains (Kittitas County). Populations of non-serpentine soil types came from various sites.

Serpentine populations of *Lupinus laxiflorus* Dougl.* (cf. var. *laxiflorus*) were

* Nomenclature according to HITCHCOCK & CRONQUIST (1976).

sampled in the drainage basin of the North Fork of the Teanaway River (Long Pass trail, Iron Peak trail, *table 1*). Individuals of non-serpentine populations of *L. laxiflorus* were collected in Haney Meadow (south of Swauk Pass) and in a plantation of Yellow pine along Bullfrog Road, west of Cle Elum.

On the summit of Iron Peak trail *L. lepidus* Dougl. var. *lobbii* (Gray) Hitchc. was encountered growing on serpentine. Non-serpentine individuals of this species were collected near Blue Mountain (Deer Park-Olympic Peninsula).

In addition, individuals of *L. polyphyllus* Lindl. (cf. var. *burkei* (Wats) Hitchc.) which were probably growing on serpentine, came from a heather-type of vegetation south of Mt. Hawkins. Non-serpentine individuals of *L. polyphyllus* were also collected in the Haney Meadow area.

Due to drought and early blooming it was only possible to find seeds in small quantities.

2.2. Soil analyses

From the Lupine-habitats visited, samples of the substrate were taken, air-dried and sieved. The fraction < 1.7 mm was used for analysis.

Exchangeable calcium and magnesium were determined by EDTA-titration of an ammonium acetate extract according to Circular 757 of the US Department of Agriculture (1947). The pH determinations were made with a glass-Ag/AgCl electrode system in a soil suspension obtained by stirring 20 g of air-dry soil and 20 ml of distilled water for an hour. The analyses were made in duplicate.

2.3. Rhizobium isolation

Excised nodules were rinsed under running water, surface-sterilized with 5 ml 1 % sodium hypochlorite (commercial preparation "Bleach" 1 : 5 diluted with water) for 7.5 minutes, rinsed with 5 ml sterilized distilled water, soaked in 5 ml 95 % ethanol for 7.5 minutes, and finally rinsed with 5 changes of 5 ml distilled water.

The nodules were subsequently homogenized in 1 ml sterile mannitol glutamate medium (VINCENT 1970). The pH was adjusted with NaOH to 7.

Autoclaving occurred at 120 °C for 15 minutes. Dilution series in a range up to 10^{-2} were made and the bacteria were plated out on solidified medium (15 g agar per liter).

2.4. Seed coat sterilization

Seeds were surface-sterilized with 95 % ethanol followed by 1 % sodium hypochlorite. Each treatment lasted 5 minutes. Subsequently the seeds were thoroughly rinsed with at least 5 changes of sterile distilled water.

2.5. Assessment of nodulation and nitrogen fixation

In order to establish whether or not *Rhizobium lupini* had been isolated from the excised nodules, *L. angustifolius*, *L. albus* L., and *L. polyphyllus* dwarf variety "Minarette" were raised as hosts under bacteriologically controlled conditions by using the modified Leonard "bottle-jar" assembly (VINCENT 1970).

The nutrient solution in the jar contains either a complete mixture (nitrate

controls) or a mixture lacking nitrate (HEWITT & SMITH 1975). The whole units were covered with aluminum foil and autoclaved at 120°C for 3 hours.

Just before planting of sterile seeds or seedlings, the top half of the aluminum foil cover was removed and the Petri dish half lifted. 3 Seeds of *L. angustifolius* or *L. albus* were planted per assembly. In the case of *L. polyphyllus*, 3 seedlings were planted. The designs were placed in the laboratory at a temperature regime of 23° to 27°C and were given overhead illumination with cool white TL 33 tubes for 16 hours each day.

Seven days after seed or seedling planting, inoculations were made by pipetting in, at a depth of 5 cm, 2 ml of a non-standardized inoculum in 0.1% $CaCO_3$ containing moderately coarse, well-washed river sand. Inocula were prepared by homogenizing agarplate-grown colonies of various strains of *Rhizobium lupini* in basal liquid medium. Finally, the river sand was covered by a 3 cm layer of sterilized gravel, grain-size 4 mm.

The nutrient solution in the jars was replenished with sterile solution when necessary. Each test was replicated three times.

To check the suitability of the above method, strains of *R. lupini* raised commercially for agricultural purposes were included. They were obtained from the Nitragin Co., Inc., Milwaukee, Wisconsin, through the courtesy of Dr. Burton, director of research.

R. lupini isolated from *L. angustifolius*, *L. albus* and *L. luteus*, labelled as 96B9, 96A5 and 96E3, respectively, are all effective in fixing atmospheric nitrogen on Blue, White and Yellow lupines. These strains induce nodules on the roots of *L. polyphyllus*, but are ineffective in fixing atmospheric nitrogen. *R. lupini* isolated from *L. polyphyllus*, labelled as 96G2, is only effective on *L. polyphyllus*.

2.6. Growth of *Rhizobium lupini* in liquid medium

The effect of different concentrations (up to 100 ppm) of nickel and chromium on the growth of some strains of *R. lupini* was traced by adding different amounts of a stock solution of $NiSO_4 \cdot 6H_2O$ or $Na_2Cr_2O_7 \cdot 2H_2O$ to the basal liquid medium. Autoclaving gave neither precipitation nor a drop in pH.

In the case of tracing the effect of different concentrations (up to 400 ppm) of magnesium, the basal medium was autoclaved separately from the stock solution of $MgSO_4.7H_2O$. Preliminary experiments indicated that autoclaving of a mixed medium caused a drop in pH. Therefore, prior to autoclaving, the pH of the basal medium was adjusted to a value just above 7. After mixing both autoclaved solutions, the pH of the test solution always reads 7.

Growth in screw cap test tubes at 28°C was monitored by changes in optical density (O.D.) at 650 nm. The growth rate is reported as Δ O.D. (STEINBORN & ROUGHLEY 1975). A growth period of 8 days was adopted for the strains 96E3 (*L. luteus*), A1 (serpentine *L. laxiflorus*) and G (non-serpentine *L. lepidus* var. *lobbii*); 14 days for the strains DI (non-serpentine *L. laxiflorus*), F (non-serpentine *L. laxiflorus*) and DII (serpentine *L. laxiflorus*); and 18 days for the strain C (serpentine *L. lepidus* var. *lobbii*). Because of these different growth periods, and the fact that the initial inoculum was not standardized, the values of

ΔO.D. are not directly comparable between the strains.

3. RESULTS

Soil analyses reveal (*table 1*) that the pH of all the localities visited does not differ substantially. The areas of suspected serpentine nature have much higher exchangeable magnesium levels (2.13–3.57 me/100 g of soil) than those that are non-serpentine (0.24–0.52 me). It seems that the exchangeable calcium level of the former (0.30–1.17 me/100 g of soil) is lower than that of the latter (1.02–2.10 me). Therefore, it is legitimate to claim that serpentine as well as non-serpentine areas in Washington have been visited and were sampled for perennial *Lupinus* species.

Regardless of the substrate, individuals of *L. lepidus* var. *lobbii* and *L. polyphyllus* are easy to excavate due to their shallow root systems. Those of *L. laxiflorus* however, can be excavated only with great difficulty and never complete, due to the depth of rooting in the rocky substrate. All root systems had nodules; serpentine and non-serpentine populations have the same number of root nodules per plant. The root systems of *L. lepidus* var. *lobbii* and *L. poly-*

Table 1. Analysis of soils of locations in Washington where *Lupinus* species have been found.

location	description	species	pH	Ca**	Mg**	Ca/Mg ratio
*along Long Pass trail	talus, sparse in grasses, herbs and shrubs; some trees	*L. laxiflorus*	6.2	1.17	2.13	0.55
*along Iron Peak trail	peridotite, sparse in herbs and grasses; a few trees	*L. laxiflorus*	5.9	0.30	3.50	0.09
*Mt. Hawkins	talus, a few herbs, *Poa curtifolia*	*L. laxiflorus*	6.3	0.31	3.00	0.10
*top of Iron Peak trail	peridotite, sparse herb and grass cover; no trees	*L. lepidus* var. *lobbii*	6.8	0.32	3.57	0.09
*Mt. Hawkins	flat, dense vegetation cover of grasses and herbs	*L. polyphyllus*	6.1	0.47	2.65	0.18
Bullfrog Road west of Cle Elum	sandstone, *Pinus ponderosa* plantation	*L. laxiflorus*	6.0	2.03	0.28	7.25
along Iron Peak trail	talus, greenstone, a few grasses and herbs	*L. laxiflorus*	5.9	1.02	0.24	4.25
Blue Mountain Deer Park	subalpine vegetation	*L. lepidus* var. *lobbii*	5.2	1.40	0.52	2.69
Haney Meadow south of Swauk Pass	coniferous forest; mixed understory Swauk sandstone	*L. polyphyllus* *L. laxiflorus*	5.5	2.10	0.37	5.68

*serpentine sites; others non-serpentine
**exchangeable cations in me per 100 g air-dry soil

phyllus possess numerous, relatively small, light-brown nodules; those of *L. laxiflorus* have only a small number of rather large, dark-brown nodules.

Plated isolates showed variation in colour and growth rates as well. After repeated plating white, opaque, sometimes gummy colonies were isolated, labelled, and finally tested.

To assess whether or not *R. lupini* had been isolated, the annual *L. angustifolius* was grown as the first host. The results (*table 2*) show that only three out of four commercially grown strains of *R. lupini* (96A5, 96B9 and 96E3) are effective in stimulating the plant to form nodules, while also being effective in fixation of atmospheric nitrogen. The commercially raised strain 96G2 as well as the strains from the wild species, were not effective in stimulating *L. angustifolius* to form nodules: the shoot dry weights were nearly indentical to the shoot dry weight of

Table 2. Effect of nitrate and N-free inoculums of various strains of *Rhizobium lupini* on mean dry shoot weight (mg) with 95% confidence interval of *Lupinus angustifolius* and *L. albus*, 37 and 57 days after seed sowing, respectively, and *L. polyphyllus* dwarf variety "Minarette", 56 days after seedling planting. Presence or absence of nodules is indicated by (+) or (—), respectively.

	Test host:	*L. angustifolius*	*L. albus*	*L. polyphyllus*
		dry shoot weight	dry shoot weight	dry shoot weight
			no inoculum added	
	uninoculated control	231 ± 8 (−)	670 ± 41 (−)	86 ± 7 (−)
	nitrate control	361 12 (−)	1393 265 (−)	1278 195 (−)
			N-free inoculum added	
strain from				
	commercial			
96A5	*L. albus*	434 8 (+)	1013 64 (+)	100 24 (+)
96B9	*L. angustifolius*	301 42 (+)	1008 39 (+)	117 23 (+)
96E3*	*L. luteus*	334 33 (+)	1015 132 (+)	184 18 (+)
96G2	*L. polyphyllus*	226 9 (−)	995 171 (+)	461 27 (+)
	non-serpentine			
DI*	*L. laxiflorus*	241 11 (−)	831 57 (+)	112 6 (+)
F*	*L. laxiflorus*	242 8 (−)	822 75 (+)	123 21 (+)
G*	*L. lepidus* var. *lobbii*	235 7 (−)	883 63 (+)	137 27 (+)
B1	*L. polyphyllus*	245 12 (−)	642 70 (+)	156 30 (+)
B2	*L. polyphyllus*	255 11 (−)	728 35 (+)	159 3 (+)
	serpentine			
A1*	*L. laxiflorus*	244 9 (−)	1010 103 (+)	119 3 (+)
A2	*L. laxiflorus*	240 9 (−)	968 197 (+)	129 17 (+)
DII*	*L. laxiflorus*	242 13 (−)	927 42 (+)	185 38 (+)
C*	*L. lepidus* var. *lobbii*	288 14 (−)	681 102 (+)	185 8 (+)
E1	*L. polyphyllus*	235 8 (−)	791 75 (+)	362 74 (+)
E2	*L. polyphyllus*	243 10 (−)	805 157 (+)	378 50 (+)

* used for growth tests in liquid medium

the uninoculated nitrate-free control. Thus, it is still doubtful whether the isolated bacteria are strains of *R. lupini*.

The results of the control experiments, as well as the effectiveness of the commercially grown strains of *R. lupini*, show that the experimental method is suitable.

For the next experiment we used the annual *L. albus* as a host. The second column of *table 2* indicates that neither the uninoculated, nitrate-free control nor the nitrate control have nodules on the root. All the inoculated individuals, however, possess root nodules. This proves that all bacteroïds isolated from the nodules sampled in the field belong to the cross-inoculation group *R. lupini*. The effectiveness of atmospheric nitrogen fixation differs substantially between the strains. There are no indications that the serpentine strains are less productive than the non-serpentine strains. Dry weights of nitrate-fed individuals are higher than those of effectively nodulated individuals.

L. polyphyllus, a perennial, was used as third and last host. The third column of *table 2* shows that both controls do not possess nodules, while the inoculated plants are effectively nodulated. The effectiveness of atmospheric nitrogen fixation, however, varies considerably. The strains isolated from *L. polyphyllus* (96G2, E1 and E2) are significantly more effective than the strains isolated from other *Lupinus* species. The highest shoot dry weight is found for the nitrate-fed plants.

For the last series of experiments, intended to establish whether or not serpentine and non-serpentine *R. lupini* strains differ physiologically, a selection has been made. Selected strains are 96E3, DI, F, G, AI, DII and C (*table 2*).

The effect of different nickel concentrations on the growth of the strains in liquid medium is presented in *fig. 1*. It seems that a small amount of nickel (0.001 ppm) results in a stimulation of growth of nearly all strains. Increased concentrations have effects depending on the strain. At 100 ppm Ni growth of the non-serpentine strains is totally inhibited. Growth of the serpentine strains is only slightly affected as compared to the Ni-free control. Strain C (isolated from serpentine *L. lepidus* var. *lobbii*) did not grow at all. This stands in contrast to the inoculation response as presented in *table 2*.

Growth as affected by different chromium concentrations is the same for serpentine and non-serpentine strains tested (*fig. 2*). There are no indications that a low concentration of chromium has a beneficial effect. Increasing concentrations lead towards a decrease of growth. At the highest concentration (100 ppm) there is no, or only a very limited, growth.

Although fresh colonies of strain C on agar were used, no growth in liquid medium occurred. In addition, strain F (non-serpentine *L. laxiflorus*) behaves similarly.

Fig. 3 shows the results of the effect of different magnesium concentrations up to 400 ppm. A small quantity of magnesium is beneficial to the growth of all the strains. The standard liquid medium, as used in both former experiments, contains 19.5 ppm Mg. Higher concentrations affect only the growth of non-serpentine strains in a negative way. At 400 ppm growth is negligible. However,

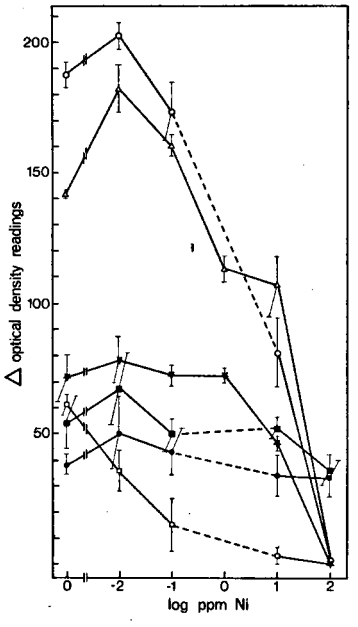

Fig. 1. Effect of different nickel concentrations on the growth of various strains of *Rhizobium lupini* in liquid medium.
✳ strain (96E3) from *Lupinus luteus*; ○ strain (DI) from *L. laxiflorus* (non-serpentine); □ strain (F) from *L. laxiflorus* (non-serpentine); ● strain (A1) from *L. laxiflorus* (serpentine); ■ strain (DII) from *L. laxiflorus* (serpentine); and △ strain (G) from *L. lepidus* var. *lobbii* (non-serpentine).
The values shown are the means with 95% confidence intervals of 3 replicates.

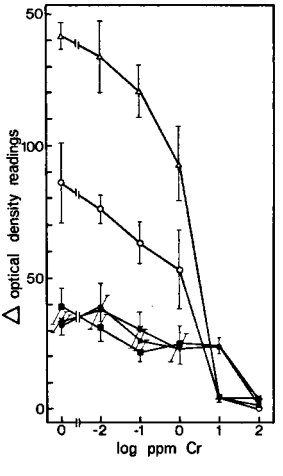

Fig. 2. Effect of different chromium concentrations on the growth of various strains of *Rhizobium lupini* in liquid medium. Legend viz. *fig. 1.*

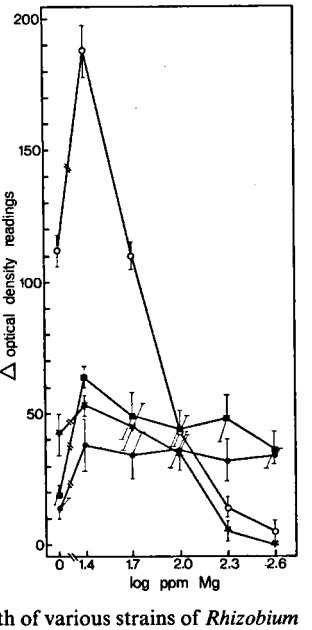

Fig. 3. Effect of different magnesium concentrations on the growth of various strains of *Rhizobium lupini* in liquid medium. Legend viz. *fig. 1.*

at this range of magnesium concentration the serpentine strains show roughly indentical growth rates regardless of the concentrations tested.

Notwithstanding each time fresh colonies were picked up from agar plates, the strains C, F and also G (non-serpentine *L. lepidus* var. *lobbii*) showed no growth.

It seems justifiable to conclude that serpentine and non-serpentine *R. lupini* differ physiologically as far as Ni and Mg are concerned. At relatively high concentrations (100 and 400 ppm, respectively) serpentine strains show no or only a slight reduction of growth. Growth of non-serpentine strains is nearly totally inhibited at these concentrations. Therefore, the serpentine strains are tolerant of higher nickel and magnesium levels than the non-serpentine ones. This response was not observed when Cr is added to the liquid medium.

4. DISCUSSION

The fact that in the Wenatchee Mountains *Lupinus* species are rare on serpentine is not caused by the absence or paucity of nodules on the root systems. It is observed that there are no ecotypic differences of *Lupinus laxiflorus*, *L. lepidus* var. *lobbii* and *L. polyphyllus* with respect to nodulation of their root systems.

Bacteroïds of *Rhizobium lupini* are present in the nodules of the three species as shown by the inoculation experiments with *L. albus* and *L. polyphyllus*. Associations between some *R. lupini* strains and these plants are characterized by effective nodulation but ineffective atmospheric nitrogen fixation. Most commonly however, there is atmospheric nitrogen fixation (*table 2*), but the quantities fixed are variable and generally low among the apparently normal associations. They failed to infect the roots of *L. angustifolius*. This points to the existence of conditioned resistance to host genotype infection by *R. lupini* for many strains within this compatible cross-inoculation group since three out of four commercially grown strains are effective in stimulating the plant to form nodules, while also being effective in fixation of atmospheric nitrogen.

It was not possible to perform such experiments with the three wild occurring *Lupinus* species because only a small quantity of seeds were available. The question whether there is effective nitrogen fixation under natural environmental conditions is still open.

Both calcium and magnesium are necessary for obtaining maximum growth rates of *R. trifolii* in liquid medium. Shortage of calcium, in the presence of sufficient magnesium, causes a reduction in growth rate. On the other hand, shortage of magnesium when calcium is sufficient, is without effect on growth rate. In addition, there is a need for having divalent cations present in a total concentration of 0.4–0.6 mM (VINCENT 1962). Increasing levels of magnesium associated with a constant level of calcium favour growth of serpentine strains of *R. lupini* (*fig. 3*). It follows that these strains are not sensitive to the total concentrations of both magnesium and calcium. Such a result is also reported (STEINBORN & ROUGHLEY 1975) for *R. meliloti* strain SU47, at all concentrations of the divalent cations (30, 60 and 130 mM) tested. Non-serpentine strains of *R. lupini* obviously react differently. The poorer growth of these strains at an

increasing total concentration of ions could be caused by toxicity of magnesium, increased amounts of sulphate, or changes in water potential. At the highest magnesium concentration (16.7 mM) these strains failed to grow. *R. trifolii* strain TA1 and *R. spec.* strain CB81 were, however, not affected by Mg/Ca ratios ranging from 30/1 to 1/1 (STEINBORN & ROUGHLEY 1975). So it seems that the effect of different Mg/Ca ratios on the growth of *Rhizobium* does not depend only on strains from other cross-inoculation groups, but also on specific strains or group of strains within a compatible inoculation group.

The growth of serpentine strains of *R. lupini* in liquid medium is not materially affected by relatively high concentrations of nickel (*fig. 1*). These strains differ physiologically from the non-serpentine strains since the latter show no growth at these concentrations. At relatively high concentrations of chromium all the strains of *R. lupini* tested show no growth in liquid medium (*fig. 2*). It might be that the ionic form in which chromium is supplied, plays a decisive role. This would mean that the anion is much more toxic than the cation (FINCK 1976) which contrasts the suggestion made by PROCTOR (1971).

The evidence obtained could mean that natural selection has produced specific strains of *R. lupini* adapted to serpentine soils. The remarkable characteristic of tolerance to one metal in living organisms is its degree of specificity. Plants and apparently also microorganisms which are tolerant of a particular ion are rarely found to be co-tolerant of ions of similar dimensions and chemical and physical properties which are absent from the soils on which they grow (TURNER 1969). Nickel and magnesium have similar ionic sizes and thus co-tolerance is not impossible, yet all sites with a high magnesium content do not necessarily support races of *Agrostis canina* and *A. stolonifera* with a high nickel tolerance and vice versa (PROCTOR 1971). Nevertheless different individual tolerances can occur together and this is correlated with the occurrence of several metals together in toxic amounts in the soil of the original habitat. PROCTOR (1971) showed that races of *A. canina* and *A. stolonifera* from serpentine soils possess multiple tolerance of magnesium and nickel, and to a lesser extent of chromium. In conclusion it seems probable that genetical adaptation of microorganisms to the serpentine habitat is analogous to the adaptation for plant species (ANTONOVICS et al. 1971). The rarity of *Lupinus* species on serpentine soils in Washington therefore does not seem to be attributable to the inability of *R. lupini* to survive in the serpentine habitat.

ACKNOWLEDGEMENTS

I wish to express my gratitude to the following members of the Botany Department of the University of Washington (Seattle, USA): to Dr. A. R. Kruckeberg for offering me the opportunity to work on this serpentine topic, and for the helpful excursions and discussions; to Dr. R. B. Walker for his advice and help; and to Dr. B. J. D. Meeuse for providing laboratory facilities and improving the English text. I am also indebted to two members of the Department of Microbiology and Immunology of the same university: Dr. G. Nester for providing laboratory facilities, and Frank F. White for his skilful cooperation with respect to isolation and testing of *Rhizobium lupini*. I appreciate critiques on the manuscript from Dr. L. K. Wiersum.

I gratefully acknowledge financial support in the form of a NATO Science Fellowship from the Netherlands Organisation for the Advancement of Pure Research (ZWO).

REFERENCES

ANTONOVICS, J., A. D. BRADSHAW & R. G. TURNER (1971): Heavy metal tolerance in plants. *Adv. Ecol. Res.* **7**: 1–85.

BIRRELL, K. S. & A. C. S. WRIGHT (1945): A serpentine soil in New Caledonia. *N.Z.J. Sci. and Techn.* **27A**: 72–76.

FINCK, A. (1976): *Pflanzenernährung in Stichworten.* Verlag Ferdinand Hirt, Kiel.

HEWITT, E. J. & T. A. SMITH (1975): *Plant mineral nutrition.* The English University Press Ltd., London.

HITCHCOCK, C. L. & A. CRONQUIST (1976): *Flora of the Pacific Northwest.* Univ. of Washington Press, Seattle and London.

HUNTER, J. G. & O. VERGNANO (1952): Nickel toxicity in plants. *Ann. appl. Biol.* **39**: 279–284.

— & — (1953): Trace elements toxicities on oats plants. *Ann. appl. Biol.* **40**: 761–777.

JOHNSON, C. M., G. A. PEARSON & P. R. STOUT (1952): Molybdenum nutrition of crop plants. *Plant and Soil* **4**: 178–196.

JONES, M. B., W. A. WILLIAMS & J. E. RUCKMAN (1977): Fertilization of Trifolium subterraneum growing on serpentine soils. *Soil Sci. Soc. Amer. J.* **41**: 87–89.

KRUCKEBERG, A. R. (1951): Intraspecific variability in the response of certain native plant species to serpentine soil. *Amer. J. Bot.* **38**: 408–419.

— (1954): Plant species in relation to serpentine soils. *Ecology* **35**: 267–274.

— (1964): Ferns associated with ultramafic rocks in the Pacific Northwest. *Amer. Fern. J.* **54**: 113–126.

— (1967): Ecotypic response to ultramafic soils by some plant species of northwestern United States. *Brittonia* **19**: 133–151.

— (1969): Plant life on serpentine and other ferromagnesian rocks in northwestern North America. *Syesis* **2**: 16–114.

MADHOK, O. P. & R. B. WALKER (1969): Magnesium nutrition of two species of Sunflower. *Pl. Physiol.* **44**: 1016–1022.

MAIN, J. (1974): Differential responses to magnesium and calcium by native populations of Agropyron spicatum. *Amer. J. Bot.* **61**: 931–937.

MASON, H. L. (1946): The edaphic factor in narrow endemism. II. The geographic occurrence of plants of highly restricted patterns of distribution. *Madroño* **8**: 241–257.

PROCTOR, J. (1971): The plant ecology of serpentine. III. The influence of a high magnesium/calcium ratio and high nickel and chromium levels in some British and Swedisch serpentine soils. *J. Ecol.* **59**: 827–842.

— & S. R. J. WOODELL (1975): The ecology of serpentine soils. *Adv. Ecol. Res.* **9**: 255–366.

RUNE, P. (1953): Plant life on serpentines and related rocks in the north of Sweden. *Acta phytogeogr. suec.* **31**: 1–139.

SASSE, F. (1979): Untersuchungen an Serpentinstandorten in Frankreich, Italien, Österreich und der Bundesrepublik Deutschland. I. Bodenanalysen. *Flora* **168**: 379–395.

SOANE, B. D. & D. H. SAUNDER (1959): Nickel and chromium toxicity of serpentine soils in Southern Rhodesia. *Soil Sci.* **88**: 322–330.

STEINBORN, J. & R. J. ROUGHLEY (1975): The effect of the ratio of magnesium : calcium on numbers of Rhizobium medium. *J. Appl. Bact.* **39**: 213–216.

TURNER, R. G. (1969): Heavy metal tolerance in plants. In: *Ecological aspects of the mineral nutrition of plants* (ed. I. H. RORISON). Blackwell Scientific Publications, Oxford. pp. 339–410.

VERGNANO, O. & J. G. HUNTER (1953): Nickel and cobalt toxicities in oats plants. *Ann Bot.* **17**: 317–328.

VINCENT, J. M. (1962): Influence of calcium and magnesium on the growth of Rhizobium. *J. gen. Microbiol.* **28**: 653–663.

— (1970): A manual for the practical study of root-nodule bacteria. *IBP handbook* **15**. Blackwell Sci.

Publ. Oxford and Edinburgh.
WALKER, R. B. (1948): Molybdenum deficiency in serpentine barren soils. *Science* **108**: 473–475.
— (1954): Factors affecting plant growth on serpentine soils. *Ecology* **35**: 259–266.
WARING, R. H. (1969): Forest plants of the Eastern Siskiyous: Their environment and vegetational distribution. *Northwest Sci.* **43**: 1–17.
WHITE, C. D. (1967): Absence of nodule formation on Ceanothus cuneatus in serpentine soils. *Nature (London)* **215**: 875.
WHITTAKER, R. H. (1954): The vegetational response to serpentine soils. *Ecology* **35**: 275–288.
— (1960): Vegetation of the Siskiyou Mountains, Oregon and California. *Ecol. Monogr.* **30**: 279–338.
WILD, H. (1974): Indigenous plants and chromium in Rhodesia. *Kirkia* **9**: 233–241.