

Influence of Soil Characteristics on the Survival of *Rhizobium* in Soils Undergoing Drying¹

WU-LIANG CHAO AND MARTIN ALEXANDER²

ABSTRACT

A study was conducted to establish whether certain soil characteristics could be correlated with the extent of survival of *Rhizobium* as the soils underwent drying. The numbers of *R. meliloti* and *R. phaseoli* fell markedly as the soils dried, but their abundance declined slowly in the soils maintained in an air-dry state. The number of surviving cells increased if these bacteria were added to sterile soil and allowed to grow before desiccation was extensive. Survival of both species was poor in soils of pH values below 5.7. The number of survivors of *R. meliloti* but not *R. phaseoli* decreased as the content of clay and of water at 15 bar suction increased. At organic matter levels below but not above 2 to 3%, the abundance of survivors of these rhizobia was related to the organic carbon content of the soil. Survival of *R. phaseoli* was inversely related to the content of total available aluminum in the soils, but the number of survivors of the two species was not correlated with the available phosphorus levels.

Additional Index Words: pH, clay content, organic matter, desiccation, aluminum, phosphorus.

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ALTHOUGH inoculation of leguminous crops with effective and competitive strains of *Rhizobium* often increases the quantity of nitrogen fixed and plant yield, the population of introduced rhizobia frequently is not maintained in numbers large enough to cause effective nodulation of the host plant in the following season. For example, the "second-year clover mortality" in Western Australia is chiefly a result of the small number of *R. trifolii* remaining in the soil after a long hot and dry summer (3,12).

The susceptibility of *Rhizobium* to drying is well established. Vincent et al. (19) showed that *R. trifolii* declined markedly during drying on glass beads or seeds. Marshall (11) later reported a more than 100- to 1,000-fold decrease in the number of *R. trifolii* when soil dried at 30°C. However, it is also known that rhizobia can survive for long periods in air-dry soil (7,18).

Marshall (11) and Bushby and Marshall (2) noted that the decline of rhizobia during desiccation was affected by soil type and clay content. In view of the apparent significance of soil drying to subsequent nodulation of legumes and the ecology of the bacteria, a study was conducted to gain information on the major soil characteristics that affect the survival of *Rhizobium* during desiccation.

MATERIALS AND METHODS

Strains of *R. meliloti* 310043, *R. meliloti* 102134, *R. meliloti* M35, *R. phaseoli* 63, and *R. leguminosarum* 128C53 that were resistant to 1.0 mg of streptomycin sulfate/ml of medium were obtained by the method of Danso et al. (4). Each strain except the last was inoculated onto their specific host plants, and the cultures used were resolated from the nodules. The bacteria were grown in 250-ml Erlenmeyer flasks containing 100 ml of selective yeast ex-

tract-mannitol (YEM) broth of the following composition: K₂HPO₄, 0.5 g; KH₂PO₄, 0.5 g; MgSO₄·7H₂O, 0.2 g; NaCl, 0.1 g; CaCl₂·2H₂O, 0.1 g; yeast extract (Difco, Detroit, Mich.), 1.0 g; mannitol, 5.0 g; streptomycin sulfate (Sigma Chemical Co., St. Louis, Mo.), 1.0 mg; and 1.0 l. of distilled water. The bacteria were grown for 6 d at 30°C on a rotary shaker operating at 120 rpm, and the cells were collected by centrifugation at 4°C and washed twice with sterile distilled water.

The soils used were collected from Jefferson County, New York, except Lima silt loam (Glossoboric Hapludalf), which was from Aurora, N.Y. The chemical and physical characteristics of the soil, particle-size distribution, and water content at 15 bar suction were determined by the methods of Greweling and Peech (8), Dower and Olson (6), and Olson (13).

Each soil was passed through a 2-mm sieve, and 1.0 ml of a washed cell suspension was added to 10 g of air-dried soil contained in 125-ml milk dilution bottles. After mixing of the soil, the bottles were incubated horizontally at 30°C and allowed to air dry. Unless stated otherwise, the period for air drying was 9 d.

At each sampling date, each soil sample was suspended in 100 ml of sterile 10% glucose solution, which was used to minimize possible osmotic shock during rehydration, and serial dilutions were made in 0.85% NaCl solution. The number of surviving rhizobia was determined by the spread-plate method on YEM agar supplemented with 1.0 mg of streptomycin sulfate and 250 µg of cycloheximide/ml. The antibiotics were sterilized by passage through Nalgene filters having a 0.2-µm pore size (Sybron Corp., Rochester, N.Y.). Three replicate soil samples were enumerated at each sampling date, and triplicate plates were prepared for each dilution. In 98% of the counts, the standard deviation of the counts was < 30% of the mean. The counts at day 0 refer to the numbers recovered from the soil.

To sterilize soil, 100-g portions contained in the dilution bottles were autoclaved for 1-h periods on each of 3 consecutive days. To ascertain whether sterility had been achieved, suspensions of the soil were plated on YEM agar. The plates were incubated for 5 d at 30°C.

RESULTS

To determine the effect of desiccation, three strains of *R. meliloti*, one strain of *R. phaseoli*, and one strain of *R. leguminosarum* were used. Cells of each strain were added to Lima silt loam, which was then allowed to dry in air. Two different phases of decline were observed (Table 1). The initial, more rapid decline in the first 9 d was followed by a phase during which little change in numbers occurred, even in a period of 75 d after the first count.

These data show that some strains are surprisingly resistant to drying in mineral soil. Such strains might be used for practical inoculum production with the carrier being a mineral soil, a material more universally available than the materials usually used as carriers for commercial *Rhizobium* inoculants. In preparing commercial inoculants, the carriers are either

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² Graduate Student and Professor of Soil Science, respectively.

Table 1—The effect of air drying nonsterile soil on the number of survivors of *Rhizobium* strains.

Bacterium	No. before drying ($\times 10^7/g$)	Survival		
		9 days	39 days	84 days
<i>R. meliloti</i> 3DOh13	1,360	4.5	2.0	2.6
<i>R. meliloti</i> 102F34	1,230	23	19	20
<i>R. meliloti</i> M3:S	860	4.5	4.5	5.9
<i>R. phaseoli</i> 6-3	1,400	2.0	1.1	0.81
<i>R. leguminosarum</i> 128C53	810	1.8	ND†	ND

† ND, not determined.

pasteurized or sterilized, and the bacteria then are allowed to grow in them. To mimic this procedure, an experiment was designed to establish whether growth of the bacteria in soil affects their resistance to drying. Four strains of *Rhizobium* were inoculated into autoclaved Lima silt loam at initial densities of about 10^7 cells/g of soil. The soil was amended with 0.1% (wt/wt) sucrose, and the moisture was maintained at 10% (v/wt). After 14 d, the soil was allowed to dry in air.

The data in Table 2 show that allowing the bacteria to grow in the soil resulted in large numbers of cells able to survive drying. Moreover, a comparison of these data with those reported above show much greater survival if the bacteria had been allowed to grow in soil. Even at day 90, the count of the three *R. meliloti* strains was about 30% of that at day 0. Because *R. meliloti* 102F34 survived well and *R. phaseoli* 6-3 survived poorly in both nonsterile and sterile Lima silt loam, they were used in further studies.

It has been reported that the resistance of introduced *Rhizobium* to drying is affected by soil properties (15). A study was thus initiated to assess the significance of soil characteristics to the resistance of rhizobia to drying. To determine the effect of pH, samples of 12 different soils with pH values ranging from 4.8 to 8.0 and clay contents varying from 24.5 to 30.5% were used. It is evident from the data in Fig. 1 that the number of surviving cells increased as the soil pH increased ($r = 0.859$, significant at the 99.9% level for *R. meliloti*; $r = 0.723$, significant at the 99% level for *R. phaseoli*). However, an inspection of the

Table 2—Number of cells surviving air drying following growth of *Rhizobium* added to sterilized soil.

Bacterium	No. at 0 day ($\times 10^7/g$)	Survival	
		9 days†	90 days†
<i>R. meliloti</i> 3DOh13	6,650	27	29
<i>R. meliloti</i> 102F34	9,800	34	34
<i>R. meliloti</i> M3:S	6,700	36	31
<i>R. phaseoli</i> 6-3	6,500	4.8	4.1

† Days after drying was initiated, which was 14 d after inoculation.

data indicates consistently poor survival of both bacteria below a pH value of about 5.7 and better survival at higher pH values, with no distinct pH effect among the more acid soils or those at higher pH values.

The influence of clay content on survival was assessed using samples of 15 different soils with clay content ranging from 1.6 to 64.0% and pH values ranging from 6.7 to 7.3. The data in Fig. 2 show that the survival of *R. meliloti* 102F34 decreased as the soil clay content increased ($r = 0.860$, significant at the 99.9% level). If the soils were separated into two groups, one with clay content <25% and the other with >40%, no correlation was evident within each group. However, the difference between the two groups was significant at the 99% confidence level. No distinct relationship was evident for *R. phaseoli* 6-3 ($r = 0.165$, correlation not statistically significant) although survivors in excess of $1.8 \times 10^7/g$ were only noted in soils with clay content of less than about 25%.

A study was conducted to determine the relation between soil organic matter level and *Rhizobium* survival during desiccation. Because the previous findings suggested that low pH and high clay content may be associated with poor survival, soils with pH values <5.5 and clay content >35% were excluded from the study. The data in Fig. 3 show that as soil organic matter increased from 0.3 to 2-3%, the survival of both rhizobia also increased. The correlations were

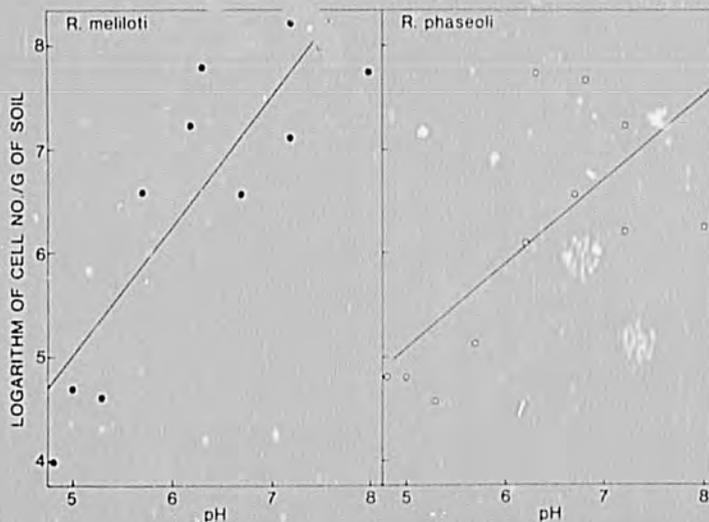


Fig. 1—Relation between survival and soil pH.

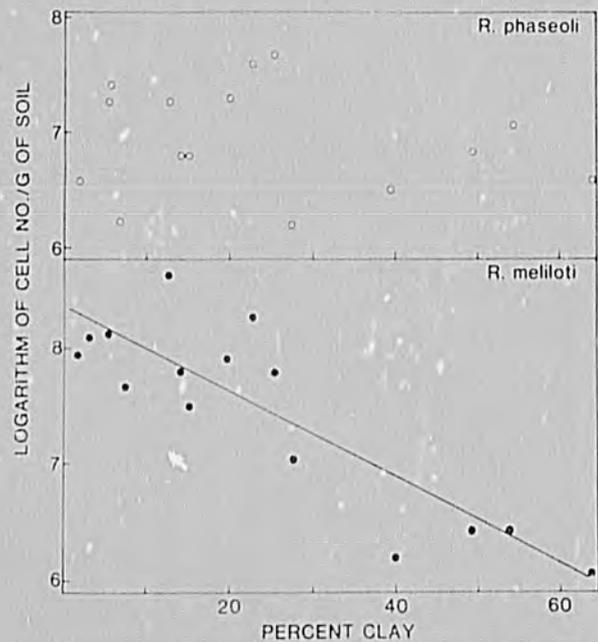


Fig. 2—Relation between survival and clay content of soil.

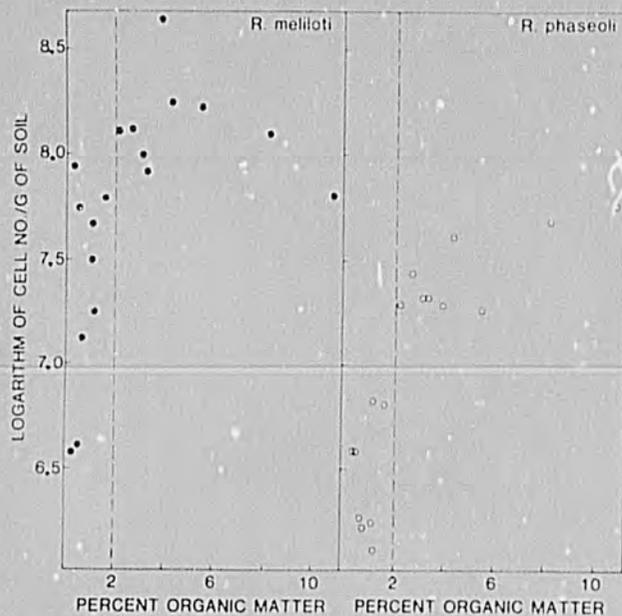


Fig. 3—Relation between survival and soil organic matter content.

statistically significant at the 95% level for both bacteria. However, in soils with organic matter content between 3 and 11%, no correlation was evident between the percent survival and organic carbon levels.

The value for soil matric suction when roots can no longer remove water from the surrounding particles is about 15 bar (1). Because a similar inability of the bacteria to compete with soil particles for water may influence rhizobial survival as soils undergo drying, an experiment was performed to study the relationship between survival and soil water content at 15 bar suction. Because organic matter imparts good water-holding capacity, soils with organic matter levels >3.0% were not studied. The data in Fig. 4 show that as water content at 15 bar suction increased, the survival of *R. meliloti* decreased ($r = 0.922$, significant at the 99.9% level). The survival of *R. phaseoli* was anomalous, increasing as the water content at 15 bar rose to about 0.05%, falling at higher levels, and then rising again.

A study was made of the possible relationship between the concentration of available P and Al and the

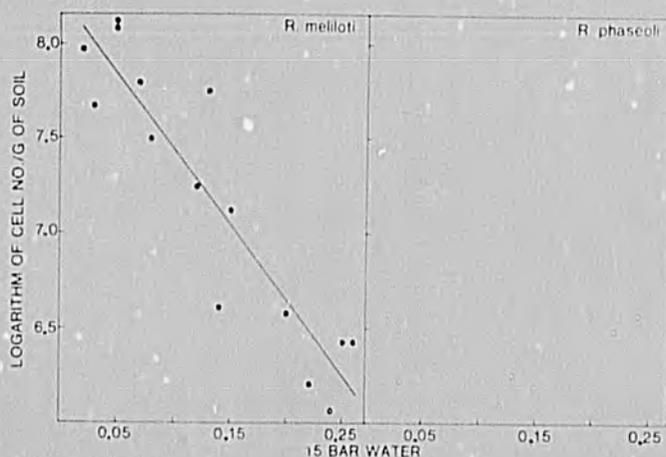


Fig. 4—Relation between survival and water content at 15 bar suction.

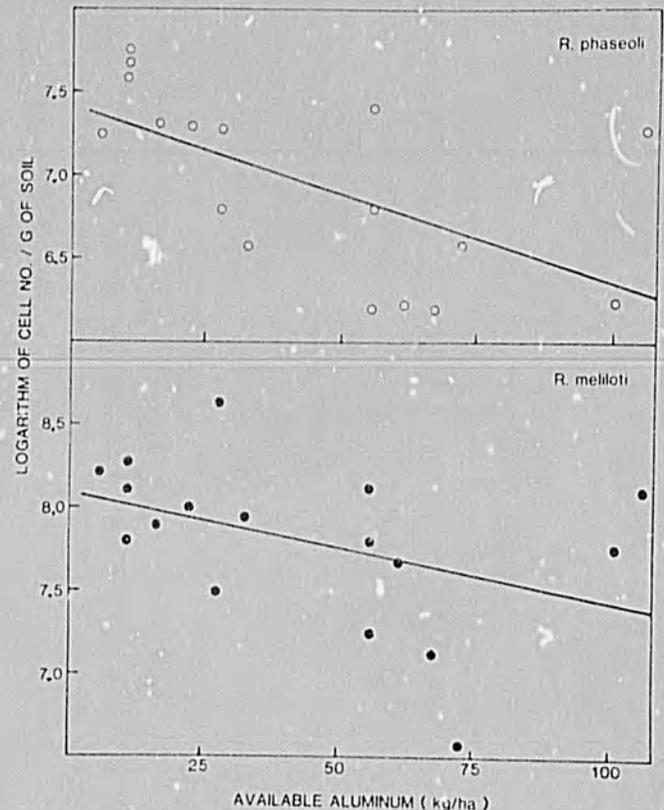


Fig. 5—Relation between survival and available Al content.

survival of *R. meliloti* and *R. phaseoli*. Seventeen different soils with pH values >6.0 and clay content <30% were used. The data in Fig. 5 indicate that as the content of available aluminum increased, the survival of *R. phaseoli* decreased ($r = 0.594$, significant at the 95% level). The relationship was not statistically significant ($r = 0.403$) for *R. meliloti*.

As indicated in Fig. 6, a relationship appeared to exist between the level of available phosphorus and the survival of both *R. phaseoli* ($r = 0.585$, significant at the 95% level) and *R. meliloti* ($r = 0.510$, significant at the 90% level). However, if the values are separated into two groups, one for soils with <5.6 kg P/ha and one for soils with >15 kg/ha, the difference between the two groups was not statistically significant. However, these data suggest that soils with less than about 3.5 kg available P/ha may sometimes be especially deleterious to the bacteria during drying.

DISCUSSION

After the initially rapid decline in *Rhizobium* abundance during drying, only a small change in population density occurs. Similar changes have been reported for species of *Rhizobium* other than those studied here (15, 19). However, the final percent of cells that survive is strain dependent. Moreover, some strains survived well in air-dried autoclaved soil but not in air-dried nonsterile soil, possibly because of a protective effect from organic materials solubilized or altered by the heat treatment or because of harmful effects of components of the indigenous microflora, such as protozoa (5), acting before the water content falls to levels too low to permit active growth or metabolism.

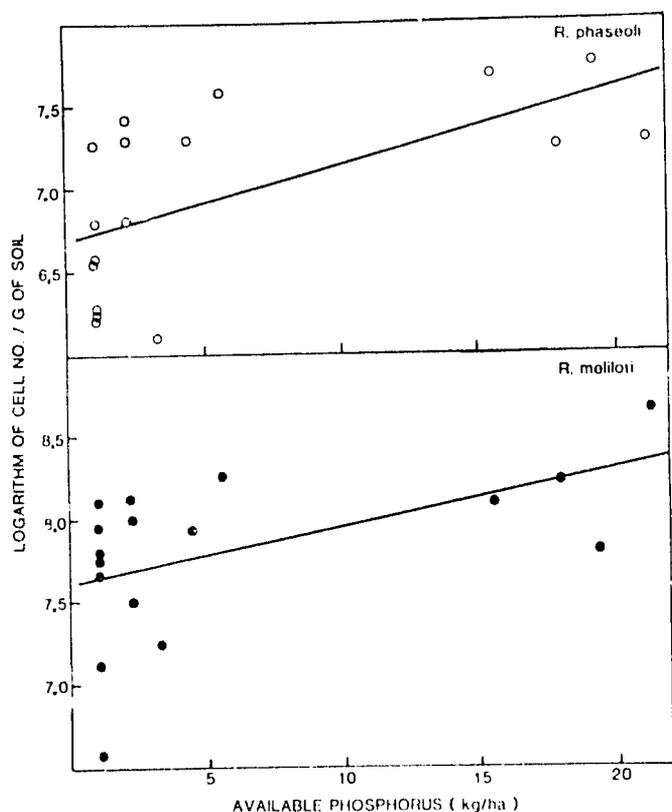


Fig. 6—Relation between survival and total available P.

Acidity is known to affect the activity of *Rhizobium* in soil (9). It is shown here that *R. phaseoli* and *R. meliloti* are particularly susceptible to drying in soils with pH value < 6.0 . This value is higher than their "critical" pH values, which are usually considered to be between 4.6 and 5.5 for *R. meliloti* and 4.0 and 5.0 for *R. phaseoli* (10). It has been reported that *R. japonicum* survived and nodulated soybeans if the plants were inoculated with air-dried soil stored for 3.5 years, provided the soil pH was > 5.8 . On the other hand, no surviving rhizobia were found if the soil pH was < 5.8 (16). Aluminum toxicity may be involved in the poor survival in acid soils (9), although none of the soils used in this investigation was highly acid.

The predominant clay in the soils used is illite ($> 40\%$), and the amount of montmorillonite is $\approx 3\%$ (K. R. Olson, personal communication). Illite has been reported to protect *Rhizobium* cells from the detrimental effects of high temperature (11). In soils with high clay contents, the percentage survival of *R. meliloti* was low. It is possible that this poor survival is related to a competition between clay particles and organisms for the limited amount of water, and the findings of a highly significant correlation between survival of *R. meliloti* and the water content of the soil at 15 bar suction is consistent with this hypothesis. On the other hand, although soil organic matter has a high water-holding capacity, no greater survival was noted with increasing soil organic matter levels ($\approx 3\%$).

Contradictory findings exist on the relationship between soil P content and the decline of rhizobia during

drying. Thus, Sen and Sen (17) found no correlation between soil P content and rhizobial survival in air-dried soil, but Pant and Iswaran (14) found a relationship between soil P and the survival of rhizobia under conditions of drying. No clear relationship was evident in the present study.

It is thus clear that characteristics of both soil and bacterium contribute to the resistance of the root-nodule bacteria to loss in viability as soils lose water. However, the finding that certain strains in some mineral soils persist in large numbers with little viability loss suggests that such mineral soils might be good carriers for inocula of root-nodule bacteria to be used in fields subjected to repeated cycles of wetting and drying.

The views and interpretations in this publication are those of the authors and should not be attributed to the Agency for International Development or to any individual acting in its behalf.

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