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Soil Acidity Tolerance of Symbiotic and Nitrogen-Fertilized Soybeans[1]

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ABSTRACT

In soybean (*Glycine max* L.), unlike other legumes, poor growth in acid soil might not be due to nodulation failure. This possibility was tested by observing lime effects on nodulation, early growth, and N concentrations in symbiotic (N_2-dependent) plants as compared with control plants fertilized with NH_4NO_3. There were two lime × N factorial trials with acid soils: a greenhouse trial in a Typic Haploxerult, with two cultivars and 13 single-strain rhizobial inoculants; and an outdoor container trial in a Typic Hapludult, with nine cultivars and three rates of inoculation that produced large differences in nodule weight and number. The soils were high in exchangeable and soluble Al. In both trials, liming from pH 4.4 to 6.0 (aqueous paste) doubled growth, regardless of N-source, cultivar, or *Rhizobium* strain or numbers; and inoculated plants were nodulated, green, and high in N even when their growth was severely acid-affected.

Symptomatic indications that soybean growth in the acid soils was limited by Al toxicity to the host plant were confirmed in solution culture experiments with pH, Al, and Ca controlled at levels resembling those found in extracts of the soil solutions. Growth was unaffected by low Ca (200 μM) or low pH (4.5), but was depressed at 30 and 65 μM Al.

The data suggest that efforts to improve acid tolerance in soybean should center on plants, not rhizobia.

Additional index words: **Legume nutrition, *Rhizobium*, Root nodulation, Nitrogen fixation, Aluminum, Calcium, Strain and cultivar selection.**

IMPAIRMENT of legume nodulation and nodule function by an environmental stress can induce N starvation and limit growth of the host plant. Conversely, impairment of host plant growth by an environmental stress can limit nodulation and N_2 fixation (8, 10). The distinction between these two cases is still ignored to the detriment of otherwise useful research (3, 4). Yet it is a simple distinction to make. In both cases there will be less plant growth, nodulation, N yield, and acetylene reduction. But if slow N_2 fixation is limiting growth there will also be evidence of N-deficiency; e.g., low N, yellow leaves, and positive response to fertilizer N. Nitrogen-fertilized control plants will be less affected than symbiotic plants if the stress primarily impairs fixation.

Soil acidity has long been known to induce N-deficiency in legumes by preventing normal nodulation. Acidity, Al toxicity, and Ca deficiency inhibit *Rhizobium* growth, root infection, and nodule activity enough to account for symbiotic failure. This information agrees with more direct evidence of N deficiency, to support the general conclusion that legumes become more sensitive to soil acidity if they depend on symbiotic N_2 fixation (9). But the conclusion might not be correct for all legumes.

An important exception might be soybean (*Glycine max* L. Merr.). This paper presents evidence that soil acidity can impair growth of soybeans equally, whether they are N-fertilized or "symbiotic" (dependent on N_2 fixation), because the effective limitation of growth and N_2 fixation is the host's susceptibility to Al toxicity. The evidence came from simple experiments comparing responses of N-supplied and symbiotic plants to acidity in two soils, with 13 strains of *Rhizobium japonicum* and nine currently commercial varieties of soybean. The importance of Al was clarified by solution culture experiments comparing the factors pH, Al, and Ca controlled at levels representing those measured in the soil solutions.

MATERIALS AND METHODS

Soybean seeds were supplied by Dr. B. H. Beard from the USDA soybean breeding project at Davis. The varieties were 'Ada', 'Amsoy', 'Clark', 'Evans', 'Grande', 'Kanrich', 'Lee', PI297550, and 'Williams'. They represent maturity groups 00, I, III, IV, and V.

Strains of *Rhizobium* were the most effective recently tested on soybean by the University of Hawaii NifTAL Project (strains CB1809, CC709, USDA110, TAL102, and the O. N. Allen strains 511, 519, 527, and 542) and by the Nitragin Co., Milwaukee (61A76, 61A101, 61A124, 61A144, and 61A150). Rhizobia were maintained on slants of yeast mannitol agar. Inoculants were made from yeast mannitol liquid cultures (barely turbid at 5 × 10^7 cells/ml), by diluting with 0.3 mM $CaCl_2$ + 0.3 mM $MgSO_4$. Th dilutions were pipetted onto seed at planting immediately before covering, and subsamples were plate-counted.

Soils and soil treatments are outlined in Table 1. We used subsoils from two California Ultisols chosen for ability to give large response to lime, N, and inoculation. Soil A, Josephine series (Typic Haploxerult), came from the foothills of the Sierra Nevada east of Sacramento. Soil B, Hugo-Josephine intergrade (Typic Hapludult), came from the Coast Range west of Sacramento. Both soils had kaolinite and oxide clay mineralogy, less than 0.1% organic matter, and no rhizobia capable of nodulating soybean. All treatments received 1 millimole of K_2SO_4/kg soil, and either 4 millimole KH_2PO_4 (Soil A) or 4 millimole $Ca(H_2PO_4)_2$ (Soil B). Zinc and Mo were supplied at 5 and 0.1 ppm, respectively. These basal fertilizer treatments and the pH amendments (Table 1) were mixed with the soil, followed by watering and a reaction period of 3 weeks (Experiment 1) or 13 weeks (Experiment 2).

Experiment 1

Growth at three pH levels was compared in Soil A. At each pH there were 13 single-strain inoculant treatments (each providing 5 to 9 × 10^6 cells/seed) and uninoculated controls without N and with NH_4NO_3 (200 mg N/pot). Triplicate pots containing 2 kg soil were sown with soybean varieties Evans and Williams; and were thinned to two plants of each variety. Pots were set in randomized blocks in large thermostat water baths, keeping soil temperature in the range 25 to 27 C. Greenhouse air temperature ranged diurnally between 22 and 32 C. Distilled water was added by weight to field capacity when required. At 42 days from sowing, plants were washed out of the soil; and shoot dry weight, nodule number, nodule weight, and percent N in shoots were measured.

Experiment 2

Growth of nine soybean varieties was compared at three pH levels in Soil B (Table 1). At each pH there was an NH_4NO_3 treatment (+N) and three rates of rhizobial inoculant. The NH_4NO_3, a single application 8 days after emergence, supplied 1.1 g N/plant. The inoculants were suspensions con-

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Table 1. Soils and soil treatments.

Soil and treatment	pH (paste)	Exchangeable† Ca	Mg	Na	K	Al	Al saturation‡	Al in 0.01 M§ $CaCl_2$ μM
Josephine soil								
11 millimol $Al_2(SO_4)_3$/kg	4.4	21.6	2.2	1.5	2.8	7.2	20	60
None	4.8	20.3	3.4	1.2	2.2	0.7	3	10
5 millimol $CaCO_3$/kg	6.2	32.8	2.9	1.4	2.2	0.0	0	0
Hugo soil								
8 millimol $Al_2(SO_4)_3$/kg	4.5	62.1	46.7	0.9	4.8	39.2	25	55
None	5.0	55.1	52.6	0.7	5.8	12.0	9	6
18 millimol dolomite/kg	6.5	84.5	79.8	0.7	5.2	0.02	0	0

† Cations extracted with 1*M* NH_4Cl at 30:1 solution:soil ratio.
‡ Exchangeable Al divided by the sum of exchangeable cations analyzed.
§ Extracted with a 10:1 solution to soil ratio.

taining equal numbers of strains CB1809 and USDA 110, grown separately and diluted to supply either 2×10^6, 2×10^5, or 2×10^4 viable cells per seed (plate count). Plants were grown in the open in early fall in crates of 650 kg soil, drip-irrigated with distilled water; two plants of each variety being interplanted in each duplicate crate. At 55 days, plants were dug and washed free of soil; and shoot dry weight, nodule number, and nodule weight were measured.

Solution Culture Experiments 3a and 3b

The separate and combined effects of pH, Al, and Ca were tested on N-supplied Clark soybean in solution cultures. The levels of each variable (Fig. 3 and Table 2) were chosen to cover the range estimated to occur in soil solution in Trials 1 and 2. Nitrogen was supplied since normally effective nodulation is difficult to achieve with soybean in solution culture and the previous experiments had already indicated that symbiotic dependence would have little effect on sensitivity to the test variables. The solutions had low P to allow maintenance of Al in solution (7) and to resemble soil solutions in this important respect. To help control of P, pH, and Al, containers were large, 20 liters. The solution, constantly aerated, contained $MgSO_4$, NH_4NO_3, and KNO_3 each 1 m*M*; either $CaCl_2$ 1 m*M* or $CaCl_2$ 0.2 m*M* with KCl 1.6 m*M*; KH_2PO_4 20 μM in 3a and 10 μM in 3b, FeEDTA 10 μM, $FeSO_4$ 3 μM, H_3BO_3 5 μM, $MnSO_4$ 1 μM, $CuSO_4$ 0.2 μM, $ZnSO_4$ 0.8 μM, Na_2MoO_4 0.2 μM, $CoCl_2$ 0.01 μM. Adjustments of pH with KOH or H_2SO_4 and of P concentration were made daily, if necessary. Aluminum was added as $KAl(SO_4)_2$ and checked weekly by analysis. Seeds were germinated on paper rolls wet with 0.2 m*M* $CaCl_2$, and transplanted at 4 days, two plants (with four plants of *Vigna unguiculata* as part of another experiment) to each triplicate pot. Plants were harvested at 25 days (Experiment 3a) or 22 days (Experiment 3b) for determination of shoot weight, root weight, and taproot length.

RESULTS

Experiment 1

Regardless of soil pH, uninoculated control plants were yellow, sparsely nodulated, stunted and low in N; inoculated plants were nodulated, green, and high in N. Some rhizobia were slightly more effective than others, but in all rhizobial treatments the plants responded alike to soil pH. Strain treatments are pooled in Fig. 1 for comparison with +N at different pH.

Soil acidity inhibited soybean growth to the same degree, whether plants were symbiotic or N-supplied (Fig. 1). At pH 4.4, leaves were slightly darker green than normal, and roots were stubby as in Al toxicity (2, 7, 13). Inoculated plants at pH 4.4 still had nodules and high N.

Experiment 2

The nine varieties behaved alike (Fig. 2a). Data for varieties are pooled in Fig. 2b, 2c, 2d for presentation of effects of inoculant rate and soil pH. These factors interacted strongly on nodule number and nodule weight (Fig. 2c, 2d); i.e., low pH and low inoculum enhanced each other's adverse effect. This strong interaction was not reflected in plant growth. Acidity affected plant growth as in Experiment 1, regardless of inoculant treatment, plant variety, or the supply of N (Fig. 2a, 2b).

Table 2. Effects of pH and Ca concentration on growth of Clark soybean in solution culture (+N).

	Shoot wg (g/plant) Ca 200 μM	Ca 1000 μM
pH 5.5	1.03	1.11
pH 4.5	1.03	1.04

Experiment 3a and 3b

Aluminum reduced shoot weight, root weight, and root length, regardless of Ca concentrations up to 1 m*M* (Fig. 3), and produced symptoms like those observed in plants grown in soil at pH 4.4. Root length, but not root weight, was low at 0.2 m*M* Ca. Neither pH nor Ca affected shoot weight (Table 2).

DISCUSSION

Soil acidity responses on two different soils were tested with nine currently used North American cultivars, three rates of inoculation, and 13 highly effective *Rhizobium* strains. None of these variables made much difference to the plants' growth response.

Poor soybean growth at low soil pH was not due to N starvation. Affected plants remained green; their leaf N concentrations were high; N fertilization did nothing to alter the response to acidity. Nodulation at low pH was sufficient to supply the N-demand of the stunted plants despite some reduction in nodule number. Failure of the soybean cultivars in these acid soils can be attributed simply to Al sensitivity of the host plant. The growth depression and the symptoms (2, 7, 13) were reproduced in N-supplied plants subjected to the appropriate Al concentration in solution culture.

If soybean can nodulate effectively in acid soil where its growth is severely limited by Al toxicity, then soybean is an exception to the generality that legumes fail in acid soil because of poor nodulation. One consequence is that it becomes justifiable to ignore rhizobia and supply N when screening for acid-soil tolerance in soybean (2, 13). The same simplifi-

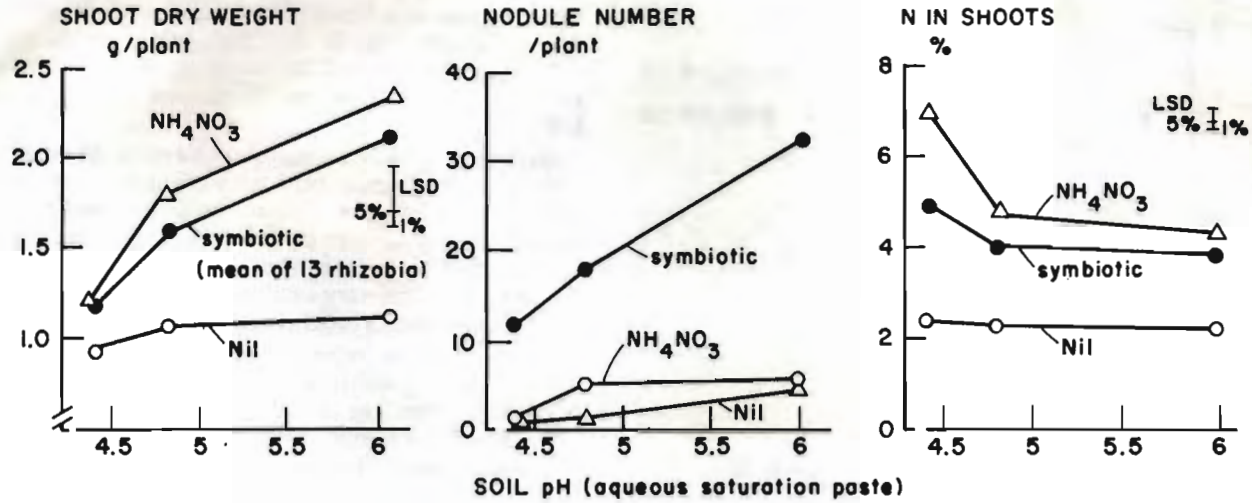

Fig. 1. Growth, nodulation, and percent N in soybean plants in Josephine soil at different pH (Experiment 1). Interactions involving plant variety and *Rhizobium* strain were not significant. Points represent pooled data for two varieties (Evans and Williams). Curves labeled "symbiotic" represent pooled data from 13 separate inoculation treatments.

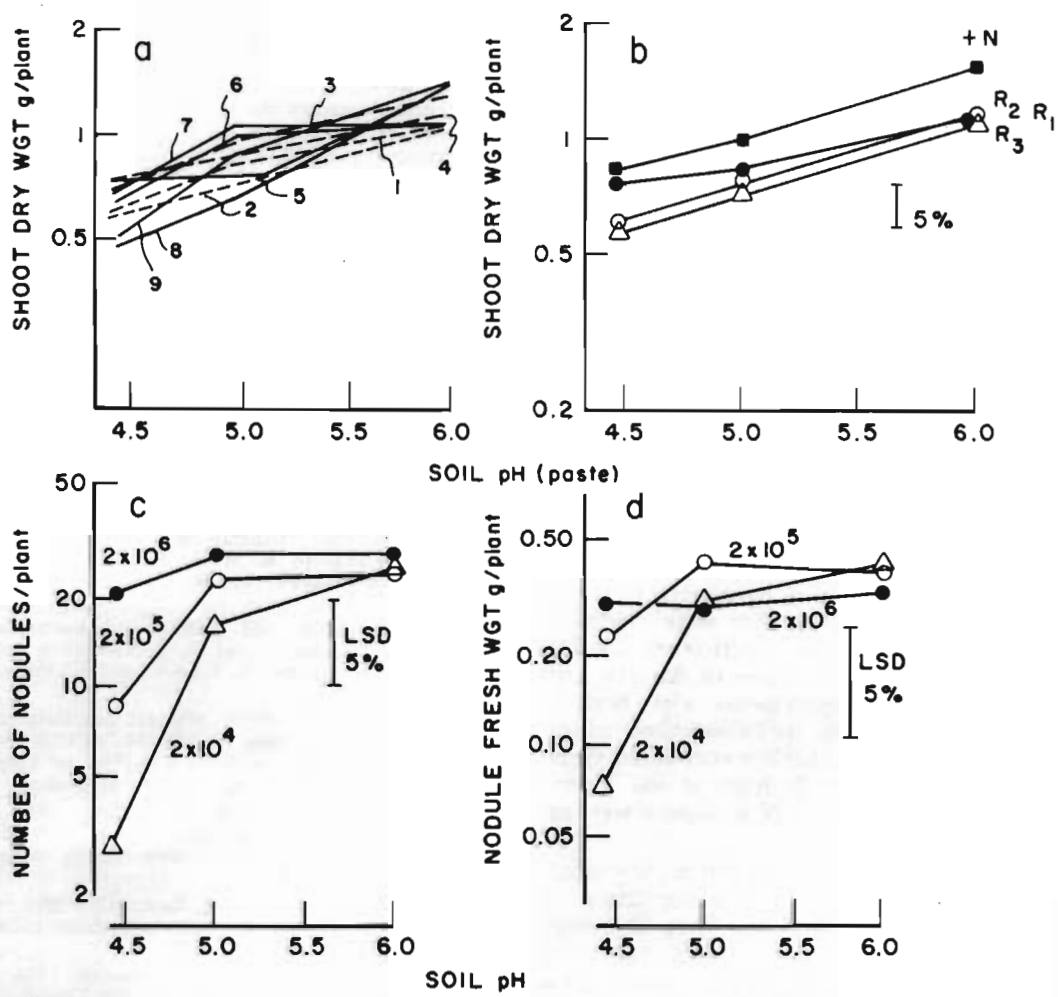

Fig. 2. Effects of soil acidity on growth and nodulation in Hugo-Josephine soil (Experiment 2). No interactions involving cultivar were significant. Accordingly, data for cultivars are pooled except in Fig. 2a. Soil pH interacted highly significantly with N/inoculant treatments on nodule number (Fig. 2c) and weight (Fig. 2d), but not on plant growth (Fig. 2b). (Cultivar numbers 1-8 in Fig. 2a represent respectively 'Ada', 'Amsoy', 'Clark', 'Evans', 'Grande', 'Kanrich', 'Lee', PI297550, and 'Williams'. Inoculant treatments R_1, R_2, and R_3 supplied respectively 2×10^4, 2×10^5 and 2×10^6 cells/seed.)

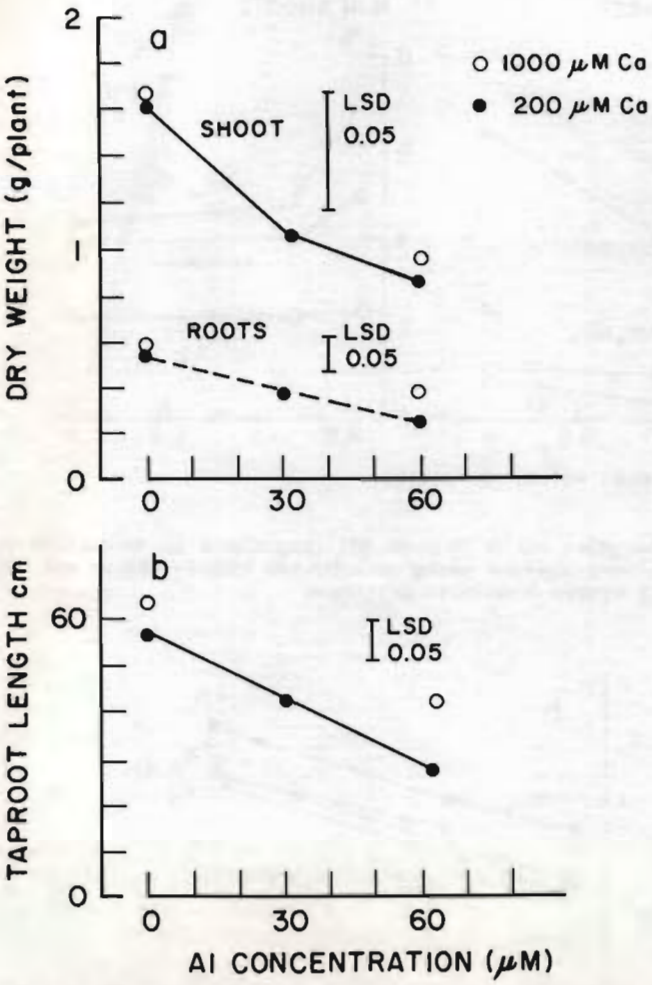

Fig. 3. Shoot and root growth by 'Clark' soybean in low-P solution culture at pH 4.5, as influenced by Al and Ca. Effect of Ca on root length (3b) is significant; interactions between Ca and Al are not.

cation is unjustified for other legumes. Nor may it be entirely justified for soybean, unless observations like ours can be generalized for different soybean types, *Rhizobium* strains, and soils.

Our findings are consistent with field trials in which large yield depressions due to acidity were evidently not accompanied by symptoms of N-deficiency (11, 14). Our data agree in detail with those of Sartain and Kamprath (12, 13) from experiments with high-Al Ultisols from North Carolina, including their observation that although nodule number correlated negatively with Al level, it was still high at the lowest soil pH (4.3 to 4.6), and percent N in shoots was unaffected.

Nevertheless, there is evidence that soil acidity sometimes causes nodulation failure and N starvation of soybean (1, 6). The experiments that show this may have involved less acid-tolerant rhizobia, very low inoculum levels, more Al-tolerant host varieties, or a type of acid-soil stress different from the Al-toxic condition imposed by Sartain and Kamprath and us. Indeed, in one case (1) the data suggest extreme Ca deficiency as the major stress component; in the other case (6) the soils were Histosols, extremely acid (pH 3.8 to 4.2) and low in Al. This might imply that results like ours should be expected only in the large group of acid soils where Al toxicity is the dominant limitation.

Soybean's ability to nodulate despite Al toxicity at pH 4.4 seems inconsistent with evidence that soybean rhizobia cannot grow well under these conditions (5). Possibly the seedlings sufficiently neutralized the rhizophere soil to protect the rhizobia from acidity and Al. Some preliminary evidence supports this hypothesis (Munns and Vonich, unpublished).

There may be other legume-*Rhizobium* symbioses in which host growth rather than the symbiotic system is critically sensitive to soil acidity. In particular, such cases should become frequent if selection of acid-tolerant rhizobia is successful.

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