Acid-Soil Resistance of Forage Legumes as Assessed by a Soil-on-Agar Method

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ABSTRACT

Establishment of small-seeded legumes on strongly acid soils can be even more difficult than maintaining those stands once established. Our objectives were to use a soil-based procedure for characterization of acid-soil resistance of small-seeded forage-legumes and to determine the validity of primary root growth and specifically the soil-on-agar technique, using germplasm that varied widely in seed size and acid-soil resistance. We ran four experiments to evaluate 28 cultivars of 15 species. In general, species differences observed were in good agreement with previous knowledge. Large differences in acid-soil resistance, for example among crimson (*Trifolium incarnatum* L.), white (*T. repens* L.), and berseem clover (*T. alexandrinum* L.) were visually obvious. Smaller differences were not as clear but could still be detected statistically; for example, the greater acid-soil resistance of striate [*Kummerowia striata* (Thunb.) Schindl.] compared with Korean lespedeza [*K. stipulacea* (Maxim.) Makino]. Relative acid-soil resistance of rose clover (*T. hirtum* All.) is reported for the first time. Within species, the known greater acid-soil resistance of 'AU Dewey' birdsfoot trefoil (*Lotus corniculatus* L.), compared with other cultivars of that species, was detected. One major difference between our results and solution culture studies, kura clover (*T. ambiguum* M. Bieb.) was more acid-soil resistant than white clover. 'Cossack', a kura clover bred from germplasm developed on a high pH soil, was more sensitive to acid-soil stress than several other cultivars. A procedure characterizing primary root growth, the soil-on-agar technique, can do an effective job of evaluating acid-soil resistance of small-seeded legumes.

Plants that are resistant to Al toxicity, or by extension acid-soil, are those, ". . . that exhibit superior root growth which ultimately result in enhanced plant vigor on acidic, Al-toxic soils or solutions" (Kochian, 1995). Aluminum tolerance and Al exclusion imply specific and different mechanisms responsible for that resistance.

Establishment of small-seeded forage species is frequently more difficult than that of larger-seeded crops. Our observations at a field site in southern West Virginia indicated that as pH dropped below 5.0, and Al availability increased, successful establishment was reduced. At a pH of 4.9, reduced numbers of 'Grasslands Huia' white clover seedlings were established and the resulting seedlings were less vigorous and more chlorotic than seedlings at pH 5.0 and above (Staley, personal communication, 2001). Observations along a pH gradient at the edge of the same field showed a dramatic drop in occurrence of white or red clover (*T. pratense* L.) when the soil pH was below 4.9 (Voigt, unpublished data, 1996). In contrast, when established from transplants, some white clover plants survived for 2 to 3 yr at a pH as low as 4.2 (Voigt, unpublished data, 1996). Thus, acid-soil resistance at seedlings stages may be even more critical than acid-soil resistance at older stages of growth.

Evaluation and selection of germplasm for resistance to acid-soil stress require simple and reliable methods. A soil-on-agar procedure, intended for use with very young seedlings of small-seeded species, was proposed for these purposes (Voigt et al., 1997). This simple, nondestructive procedure evaluates root emergence of germinated seedlings from a thin layer of soil into water-agar during a ≈10-d period. Results for white clover, based on eight soils varying widely in Al saturation, indicated a strong relationship between root emergence of white clover and the species of Al in the soil solution that are toxic to the roots of dicotyledonous plants (Voigt et al., 1998). The usefulness for the characterization of germplasm of this procedure, which is based on primary root growth, remains to be demonstrated.

The abilities of clovers to grow on acid and calcareous soils vary widely (Hoveland and Evers, 1995). Clovers that are among the more resistant to acid-soil stress, such as crimson and subterranean clover (*T. subterraneum* L.), can be among the more susceptible to iron chlorosis when grown on calcareous soils (Rogers, 1947; Gildersleeve and Ocumpaugh, 1988). In contrast, berseem clover remains green and grows well on calcareous soils (Gildersleeve and Ocumpaugh, 1988) but is not recommended for acid soils (Hoveland and Evers, 1995). Even within species, where wide genetic variation is known, germplasm that is more resistant to acid-soil stresses can be more susceptible to iron chlorosis. Osborne et al. (1981) studied the response of seven sub clover cultivars to Al in acid solution cultures. Those same cultivars were included in studies of iron chlorosis by Gildersleeve and Ocumpaugh (1989). Rank correlation coefficients calculated between Gildersleeve and Ocumpaugh's chlorosis scores at 6 wk and the relative root growth characteristics (4 mg kg^{-1}/0 mg kg^{-1} Al $\times$ 100) of Osborne et al. were $r = -0.95$ and -0.90 ($n = 7$, $P < 0.01$) for the Paritta soil (clayey, mixed, active, hyperthermic, shallow Petrocalcic Paleustolls) and root weight and the Denhawken soil (fine, smectitic, hyperthermic Vertic Haplustepts) and root length, respectively. Thus, in evaluating results from the soil-on-agar procedure with those reported in the literature, not only acid-soil and Al resistance should be considered, but also iron chlorosis resistance.

Our hypothesis was that the soil-on-agar procedure, which uses primary root growth to characterize, could be used to determine the acid-soil resistance of small-

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Abbreviations: CoV_E, coefficient of velocity of root emergence; RI, resistance index.

seeded legumes. To do this, differences in seedling vigor, frequently associated with seed-size, would have to be overcome. Results were evaluated by comparing our rankings for acid-soil resistance to rankings reported in the literature from both soil and solution-culture experiments. Further, our studies would provide an assessment of acid-soil resistance of a wide array of small-seeded legumes using a single soil-based technique. Few reports of this type are available.

MATERIALS AND METHODS

Experimental Procedure

The soil-on-agar procedure has been described (Voigt et al., 1997). Briefly, a layer of moist soil ≈8 mm deep was gently and uniformly distributed on top of solidified water agar (5 g kg^{-1} agar in distilled water) in a rectangular, clear, plastic flask. Germinated seeds, with a uniform radical length of ≈1 mm, were planted immediately below the soil surface. Flasks containing 18 seedlings each were randomized on trays, with one replication per tray, and placed in a growth chamber. Growth chamber conditions were 12 h of light, at ≈5 μmol m^{-2} s^{-1}, at 23°C and 12 h of darkness at 15°C. Root emergence from the soil into the agar was scored throughout each experiment. Observations were made every 8 h, usually starting at Hour 19 and extending through Hour 75. Less frequent observation was needed after 75 h because of reduced rates of root emergence. After each reading, to minimize any effects of variation in environmental conditions within the chamber, flask position within a tray (replication) was changed and tray positions were rotated.

Four experiments (A thorough D) were conducted during October (C) and December (D) 1997 and March (B) and September (A) 1998. Each experiment had five replications and used a single lot of throughly mixed Porters soil (fine-loamy, isotic, mesic Typic Dystrudepts), containing A and Bw horizons. Prior to an experiment, lime ($CaCO_3$) was thoroughly mixed with the soil and water was added to bring the soil to field capacity. Soil was incubated for 2 wk prior to use. At the beginning of the experiments, the low stress (control) treatment had a pH of between 5.0 and 5.2 (Al saturation of ≈6 to 14%). The low pH, high Al stress soil treatment had a pH of between 4.3 and 4.4 (Al saturation of 53 to 62%). Where a wide response range among entries was anticipated, an intermediate level, pH 4.6 to 4.7 (Al saturation of 32 to 39%) was included.

Forage legumes evaluated (Table 1) included clovers, crownvetch {*Coronilla varia* L. [= *Securigera varia* (L.) Lassen]}, lespedezas, milkvetch, and trefoil. Seed weight ranged from 60 to almost 1000 mg per 100 seed. A single lot of Huia white clover, stored at <0° C, was used as a control entry in each experiment. White clover was used as a control because of our previous experience with the species (Voigt et al., 1997, 1998).

Data Analysis

Root emergence counts for each flask were converted to a percentage of the number of germinated seed planted. Total number of seedlings was reduced by any obvious defective seedlings and by the occasional seedling whose root growth, rather than proceeding into the soil, forced the seed up above the top of the soil surface by more than ≈5 mm. Mean cumulative root emergence percentage was then analyzed.

Kotowski's coefficient of velocity (CoV_E; Scott et al., 1984) was calculated as an estimate of rate of root emergence:

$$CoV_E = 100(\Sigma N_i/\Sigma N_iT_i),$$

where N_i is the number of roots emerged at time i, and T_i is the number of hours from planting.

The number of hours to reach x% of potential emergence was determined. Potential emergence of each entry was defined as the maximum emergence of that entry for the control treatment in the same replication. The level of x% varied from 20 to 50%, depending on the experiment. An x value as close to 50% as possible was used. Species that were most sensitive to the acid soil frequently did not reach 50% of their potential emergence in the pH 4.3 to 4.4 treatment. In those cases, the same lower percentage (<50%) was used for all entries.

Table 1. Forage legumes evaluated in four soil-on-agar experiments.

Species	Cultivar	100-seed weight	Experiment
		mg	
clover, alsike (*Trifolium hybridum* L.)	'Daubiji'	67	A
	'Rausviaj'	75	A
clover, arrowleaf (*T. vesiculosum* Savi)	'Yuchi'	162	A
clover, berseem (*T. alexandrinum* L.)	'Bigbee'	305	C
	'Joe Burton'	323	C
clover, crimson (*T. incarnatum* L.)	'Chief'	593	A
	'Dixie'	613	A, B
	'Tibbee'	520	A
clover, kura (*T. ambiguum* M. Bieb.)	'Cossack'	197	D
	'Endure'	230	D
	'KZ2'	216	D
	'NF93'	220	D
	'Rhizo'	181	D
clover, red (*T. pratense* L.)	'Arlington'	205	C
	'Cinnamon'	208	C
clover, rose (*T. hirtum* All.)	'Overton R-18'	316	A
clover, subterranean (*T. subterraneum* L.)	'Mt. Barker'	1004	B
crownvetch {*Coronilla varia* L. [= *Securigera varia* (L.) Lassen]}	'Penngift'	407	B
lespedeza, Korean [*Kummerowia stipulacea* (Maxim.) Makino]	common	234	B
lespedeza, striate (*Kummerowia striata* (Thunb.) Schindl.)	'Kobe'	288	B
lespedeza, sericea (*Lespedeza cuneata* (Dum.-Cours.) G. Don)	'Serala'	173	B
milkvetch, cicer (*Astragalus cicer* L.)	common	421	B
trefoil, birdsfoot (*Lotus corniculatus* L.)	'AU Dewey'	128	C, D
	'Dawn'	121	D
	'Fergus'	107	D
	'Georgia-1'	128	D
	'Norcen'	149	D

Resistance index (RI) was calculated for each of the above variables on a within-replication basis (low or intermediate pH treatment value/control (high) pH treatment value × 100).

Data were analyzed using Proc Mixed (SAS Institute, 1997). Reps were considered random while pH treatments and entries were considered fixed. Differences between residual log likelihood estimates were compared to the χ^2 distribution to determine when separate variance groups were required for different pH treatments. Contrast statements were used to compare species, to examine species by pH interactions, and to make comparisons among cultivars within a species.

RESULTS AND DISCUSSION

We recognize that the assessment of Al resistance of a species is most appropriately determined by studying many germplasm of each species. We have not done that. Our use of species names, in place of cultivar names, is for purposes of brevity and simplicity in presentation. We have used cultivars that are widely available in the USA. In that respect, our results are reasonably representative of those species as they exist in U.S. agriculture.

Experiment A (Alsike, Arrowleaf, Crimson, Rose, and White Clover)

Root emergence for Exp. A was typical (Fig. 1). At the highest pH, where Al has little or no effect on most species, root emergence, once begun, occurred rapidly. Emergence was delayed at the lower pH levels, presumably because of the higher levels of Al in the soil solution (Voigt et al., 1998).

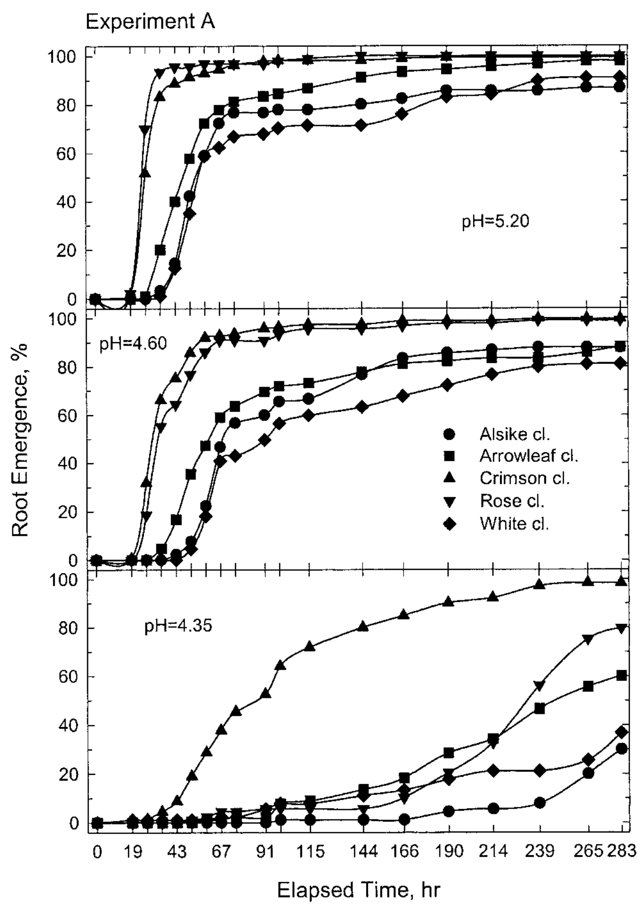

Fig. 1. Experiment A, effect of three levels of soil pH on root emergence from soil into agar across time for alsike (mean of 'Daubiji' and 'Rauvsiaj'), arrowleaf, crimson (mean of 'Chief', 'Dixie', and 'Tibbee'), rose, and white clover.

Rose clover, compared with other species in this or the other experiments, appeared to have a more critical response to soil pH. At pH 5.20 and 4.60, it emerged much like crimson clover, however, at pH 4.35 it emerged more like alsike (*T. hybridum* L.) or white clover until ≈166 hr, when its emergence began to increase rapidly. Two factors might explain this change in response. First, initial growth of very young seedlings, from 24 up to 72 h, is dependent on reserves in the seed and not on nutrients from the soil (Edmeades et al., 1995) and is expected to be more closely related to Al toxicity than is later growth. Second, although our limited analyses of the pH of soil removed from the agar have not been entirely consistent, we have observed that the pH of the most acid soil can increase by up to 0.03 pH units d^{-1} during a soil-on-agar experiment. Because of both of these factors, the response of seedlings to the soil-on-agar system would be expected to change with time. The response of rose clover to the pH 4.35 soil treatment after 144 to 166 h, while probably a reflection of both factors, is likely related more to pH changes than to differences associated with exhaustion of endosperm nutrients. To minimize the impact of potential pH changes, observations later than 166 hr were not included in calculations of mean cumulative root emergence or CoV_E.

Species differences in root emergence (Table 2) were detected at all pH levels and species × pH interactions were statistically significant ($P < 0.01$). Paired interaction analysis indicated that most species differed in their response to pH. Emergence of all species declined more between pH 4.60 and 4.35 than between 5.20 and 4.60, a nonlinear response. Alsike clover was intermediate in response compared with white, arrowleaf (*T. vesiculosum* Savi), and crimson clover. The interaction for rose clover was different from all other species because of its large decline in emergence between pH 4.60 and 4.35. The RI values differed also, with crimson and rose clover being more resistant than white clover at pH 4.60, but only crimson clover being different from white clover at pH 4.35. Results from analysis of hours to 20% emergence (data not shown) produced a somewhat different picture with alsike clover and, especially, rose clover being more sensitive at pH 4.35 than white clover. For this characteristic, rose clover was more severely impacted by acid soil then any other species.

Results of Exp. A indicate that the acid-soil resistance, and by inference Al resistance, of these species can be characterized as: Crimson clover > arrowleaf clover ≥ white clover > rose clover ≥ alsike clover. Literature indicates that arrowleaf clover is less tolerant of acid soil (Hoveland et al., 1969) but is more susceptible to iron chlorosis (Gildersleeve and Ocumpaugh, 1988) than crimson clover. This suggests that arrowleaf clover may have a narrower soil pH adaptation than crimson clover. Acid-soil adaptation of rose clover is not well defined, but rose clover is more resistant to

Table 2. Mean cumulative root emergence from soil into agar at three soil pH levels and acid-soil resistance index of clover species, Experiment A.

		Cumulative root emergence					Resistance index†				
		Soil pH					Soil pH				
Species	Cultivar	4.35	4.60	5.20	Range	Int.‡	4.35	4.60	Mean	Range	Int.
		%									
Crimson clover	Mean§	38.4A¶	79.0A	83.9A	46	C	46.0A	94.4A	70.2A	48	C
Arrowleaf clover	'Yuchi'	4.2B	46.2B	60.7B	56	B	6.5B	77.2BC	41.9B	71	AB
Rose clover	'Over-R18'	3.5B	73.4A	87.7A	84	A	3.9B	83.8AB	43.9B	80	A
White clover	'Huia'	4.4B	31.0C	45.6C	41	D	8.7B	68.8CD	38.7BC	60	BC
Alsike clover	'Daubiji'	0.1a	23.1b	44.8a	45	b	0.2a	51.5b	25.9b	51	b
	'Rausviaj'	0.3a	37.5a	50.9a	51	a	0.6a	76.1a	38.4a	76	a
	Mean	0.2B	30.3C	47.9C	48	D	0.4B	63.8D	32.1C	63	B
Overall mean		15.97	56.03	67.68			19.74	80.07	49.91		

† Resistance index = emergence (pH X/pH 5.20 × 100), where X = 4.35 or 4.60.
‡ Int. = interaction; different letters in the column indicate significant entry × pH interactions at the 0.05 probability level.
§ Crimson clover mean = mean of 'Chief', 'Dixie', and 'Tibbee'; differences among crimson clovers were not significantly different at the 0.05 probability level.
¶ Values in a column followed by the same letter (lower case for cultivar and upper case for species comparisons) are not significantly different at the 0.05 probability level by ANOVA contrast.

iron chlorosis than crimson clover (Gildersleeve and Ocumpaugh, 1988). This would lead us to predict that it should, as observed here, be less acid-soil resistant than crimson clover. Solution culture studies suggest that alsike clover is less resistant to Al than white clover (Wheeler and Dodd, 1995a,b).

We did not detect differences in acid-soil resistance among three crimson clovers cultivars, Chief, Dixie, and Tibbee (data not shown). Although no other studies of acid-soil resistance in crimson clover have been reported, Gildersleeve et al. (1988) reported that Chief was more susceptible to iron chlorosis than Dixie, a difference not mirrored in our results. We did observe a difference in RI between two alsike clovers (Table 2), although a part of this difference might be a response to the poorer seedling vigor of the less resistant cultivar, Daubiji.

Correlation coefficients between emergence and seed weight ranged from 0.89 to 0.94 for pH 5.20 and 4.35, respectively ($P < 0.01$). Root emergence was closely related to seed weight. However, our assessment of the acid-soil resistance of these clover species was in general agreement with acid or calcareous-soil resistance information in the literature. Thus, we concluded that our data are a valid assessment of acid-soil resistance and not just an indication of differential seedling vigor associated with differences in seed weight.

Experiment B (Crimson, Sub, and White Clover; Cicer Milkvetch; Crownvetch; and Korean, Sericea, and Striate Lespedeza)

Species in Exp. B ranged widely in their response to soil pH (Fig. 2). All roots emerged relatively rapidly at pH 5.24. Cicer milkvetch (*Astragalus cicer* L.), crownvetch, and white clover showed the greatest delay in emergence at pH 4.70. All species were delayed to some degree at pH 4.38, compared with pH 5.24. At this low pH, the eight species had a wide range of response. Differences among species in mean root emergence were detected at all three levels of pH (Table 3) and the interaction between treatment and species was significant ($P < 0.01$). The range in response of species to pH varied from 19 to 76% for sub clover and milkvetch, respectively. Comparisons of the responses of paired species to pH indicated that all differed except for crownvetch and milkvetch. There was a general relationship between the range of the species response and the occurrence of a significant interaction. However, this relationship is not exact because the response to pH for some species was linear and that for others was curvilinear. For example, the responses of crownvetch and milkvetch to pH were relatively linear; whereas that of Korean lespedeza, with a similar range, was curvilinear. In contrast, although striate lespedeza and white clover had a similar range of emergence and both species had a curvilinear response, that of striate lespedeza was more extreme because all its decline in emergence occurred between pH 4.70 and 4.38.

Differences in RI among species were greatest at pH 4.38. The change in RI between the two pH levels varied among species, with striate lespedeza having the greatest range and sub clover the least.

Results of Exp. B indicate that the acid-soil resistance, and by inference the Al resistance, of these species can be characterized as: sub clover > crimson clover > sericea lespedeza [*Lespedeza cuneata* (Dum. Cours.) G. Don] > striate lespedeza > Korean lespedeza ≥ white clover ≥ crownvetch ≥ cicer milkvetch. These results are in very good agreement with the literature. Both sub and crimson clover are widely recognized as highly resistant to acid-soil stress (Donnelly and Cope, 1961; Pires et al., 1992). Although we have not found studies that compare the acid-soil or Al resistance of the two clovers directly, 'Mt. Barker' sub was more severely affected by iron chlorosis than Dixie crimson clover (Gildersleeve and Ocumpaugh, 1988). This result suggests that the more chlorosis-susceptible sub clover would be expected to be more acid-soil resistant than crimson clover. Sericea lespedeza, also with excellent adaptation to acid soils (Cline and Silvernail, 1997) was reported to be more tolerant of acid soils than Korean lespedeza (Hyland, 1938). Striate lespedeza was more tolerant of acid soil and less tolerant of alkaline soil than Korean lespedeza (McGraw and Hoveland, 1995).

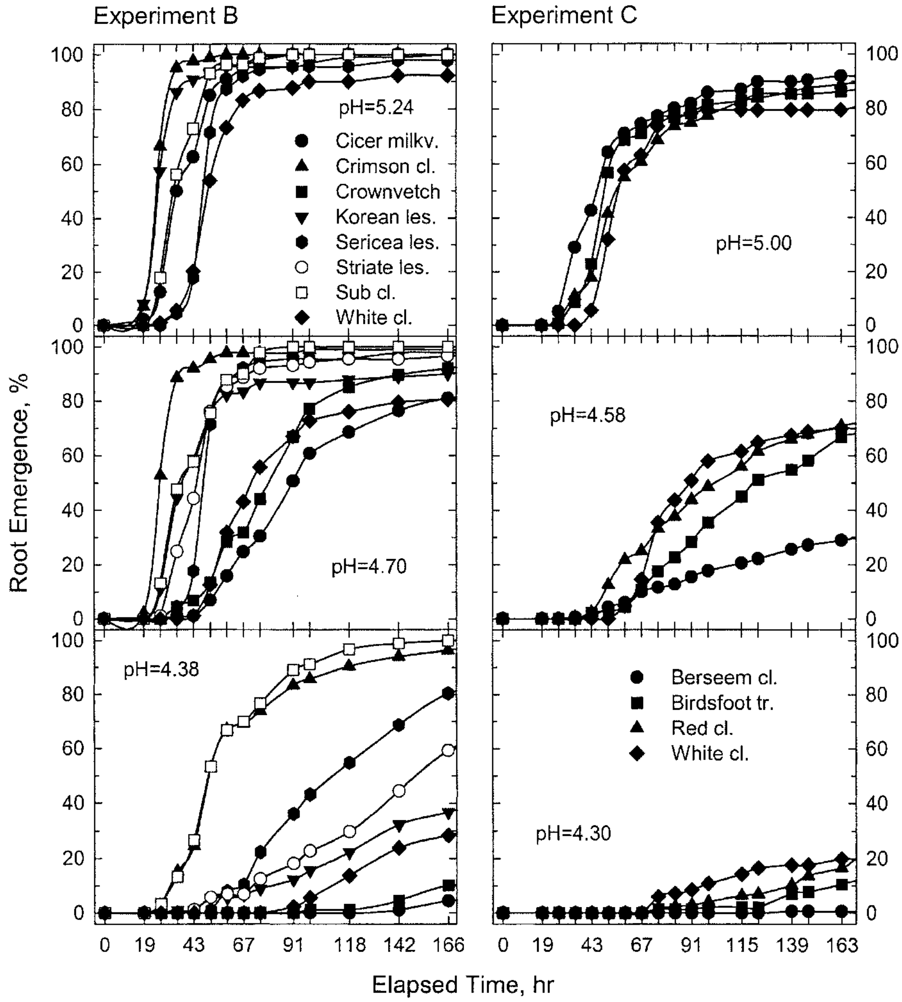

Fig. 2. Effect of three levels of soil pH on root emergence from soil into agar across time for: Exp. B, crimson, sub, and white clover; cicer milkvetch and crownvetch; and Korean, striate, and sericea lespedeza. Crownvetch and striate lespedeza are not shown for soil pH 5.24 because of overlapping plots. Experiment C, berseem (mean of 'Bigbee' and 'Joe Burton'), red (mean of 'Arlington' and 'Cinnamon'), and white clover and birdsfoot trefoil.

Crownvetch is apparently quite sensitive to acid-soil stress (Hyland, 1938; Munns and Fox, 1977). All these results are in agreement with our results.

Correlation coefficients between seed weight and mean root emergence and RI at pH 4.38 were 0.75 and 0.72, respectively ($n = 8$, $P < 0.5$). Plots of the data indicated that the relationship was caused by differences between two groups of species, the large seeded and more acid-soil resistant sub and crimson clover vs. the remaining smaller seeded species. Within the smaller

Table 3. Mean cumulative root emergence from soil into agar at three soil pH levels and acid-soil resistance index of clover, lespedeza, and small-seeded vetch species, Experiment B.

		Cumulative root emergence					Resistance index†				
		Soil pH					Soil pH				
Species	Cultivar	4.38	4.70	5.24	Range	Int.‡	4.38	4.70	Mean	Range	Int.
		%									
Sub clover	'Mt. Barker'	60.4A§	74.6B	79.4BC	19	G	76.1A	94.1AB	85.1A	18	E
Crimson clover	'Dixie'	58.1A	86.0A	89.6A	32	F	64.8B	95.9AB	80.3A	31	DE
Sericea lespedeza	'Serala'	25.0B	55.9C	65.4E	40	E	38.4C	86.9AB	62.6B	48	B
Striate lespedeza	'Kobe'	15.9C	68.2BC	68.4DE	52	D	23.8D	100.8A	62.3B	77	A
Korean lespedeza	common	11.4CD	67.9B	85.5AB	74	B	13.2E	79.4BC	46.3C	66	AB
White clover	'Huia'	5.7DE	40.0D	59.7E	54	C	9.3EF	67.5CD	38.4C	58	ABC
Crownvetch	'Penngift'	1.3E	41.5D	73.1CD	72	A	1.9FG	57.8DE	29.8CD	56	BC
Cicer milkvetch	common	0.4E	32.1D	76.7C	76	A	0.6G	41.8E	21.2D	41	CD
Overall mean		22.27	58.28	74.73			28.51	78.02	53.26		

† Resistance index = emergence (pH X/pH 5.24 × 100), where X = 4.38 or 4.70.
‡ Int. = interaction; different letters in the column indicate significant entry × pH interactions at the 0.05 probability level.
§ Values in a column followed by the same letter are not significantly different at the 0.05 probability level by ANOVA contrast.

seeded species there was no relationship between seed weight and root emergence. The relationship between seed mass and root emergence at low pH did not obscure relationships expected from previous reports of acid-soil resistance.

Experiment C (Berseem, Red, and White Clover and Birdsfoot Trefoil)

Differences in root emergence among white and red clover and birdsfoot trefoil were relatively minor when compared with that of berseem clover (Fig. 2). Few berseem clover roots emerged at pH 4.30 even when the experiment was extended beyond 300 h (not shown). At that low pH, the order of emergence was: white clover, red clover, and then birdsfoot trefoil. Differences in mean emergence among the three perennials were detected at pH 4.58 and 5.00. Birdsfoot trefoil emerged before white clover at pH 5.00 but more slowly than either red or white clover at pH 4.58 (Table 4). This was reflected in the interactions and RI among the three species. Because of the large number of zeros in the berseem data at pH 4.30, that data was not included in the statistical analysis. However, even at pH 4.58 berseem had a RI less than half that of the perennials. Red clover cultivars Arlington and Cinnamon did not differ in response to soil pH, neither did 'Bigbee' and 'Joe Burton' berseem clover (data not shown).

In a separate experiment that compared Huia white clover with 10 additional red clover cultivars at two pH levels (Voigt, unpublished data, 1998), red clover was less resistant to acid-soil stress than white clover.

Results of these experiments indicate that the acid-soil resistance, and by inference Al resistance of these species can be characterized as: white clover ≥ red clover ≥ birdsfoot trefoil > berseem clover. Berseem clover is adapted to near neutral to alkaline soils (Hoveland and Evers, 1995) and is resistant to iron chlorosis (Gildersleeve and Ocumpaugh, 1988). Solution culture studies suggest that red clover is less resistant to Al stress than white clover (Edmeades et al., 1991a,b; Wheeler and Dodd 1995a,b). Results from soil-based studies are less clear. They tend to rank red clover as similar to white clover (Baligar et al., 1985; Milan et al., 1990). In both studies, red clover was numerically less than but not statistically different from white clover.

Correlation coefficients between seed weight and other characters were not statistically significant except for a negative relationship with root emergence at pH 4.30 ($r = -0.82$, $P < 0.05$). Despite its large seed, berseem clover had the slowest root emergence at low pH.

Experiment D (Kura and White Clover and Birdsfoot Trefoil)

Birdsfoot trefoil roots emerged before those of white clover at pH 5.20, but those of kura clover emerged more quickly and much more completely than did roots of the other two species at pH 4.40 (Fig. 3 and Table 5). In contrast to Exp. C, both the species × pH interaction and the RI indicated that the acid-soil resistance of birdsfoot trefoil and white clover were similar. However, RI for CoV_E (not shown) indicated that white clover was more resistant than birdsfoot trefoil. Results from Exp. D indicate that the acid-soil resistance and, by inference, the Al resistance of these species can be characterized as: kura clover > white clover ≥ birdsfoot trefoil.

Little has been reported about the acid-soil resistance of kura clover. On the basis of solution culture studies, Wheeler and Dodd (1995a, 1995b) reported that the Al resistance of kura clover was similar to that of birdsfoot trefoil and less than that of white clover. For this species, our soil-based system produced a much different result. It is possible that this difference in results could be caused by the different stages of growth or roots studied. Our results were based on primary root growth immediately after germination while their results were based on 28 to 35 d of growth following emergence of the first true leaves. Townsend (1985) indicated that kura clover prefers noncalcareous soils, suggesting a possible adaptation to at least moderately acid soils. Bryant (1974) reported that kura clover grew on soils with a pH as low as 4.9 where white clover failed. Strachan et al. (1994) reported dominance of kura clover on plots with a pH as low as 4.9. At the very least, our results suggest that a closer look at the Al and acid-soil resistance of kura clover would be worthwhile. Kura clover, through

Table 4. Mean cumulative root emergence from soil into agar at three soil pH levels and acid-soil resistance of clover and trefoil species, Experiment C.

		Cumulative root emergence					Resistance index†				
		Soil pH					Soil pH				
Species	Cultivar	4.30	4.58	5.00	Range	Int.‡	4.30	4.58	Mean	Range	Int.
		%									
White clover	'Huia'	7.4A§	33.7A	53.9C	46	C	14.5A	63.9A	42.6A	49	A
Red clover	Mean¶	4.1A	34.3A	56.9BC	53	C	7.7A	61.4A	41.3A	54	A
Birdsfoot trefoil	'Dewey'	2.5A	24.6B	64.5AB	62	B	3.7A	39.3B	22.7B	36	A
Berseem clover	Mean	0.1nd#	12.7C	66.2A	54††	A		19.5B			
Overall mean		4.51	25.39	60.78			8.41	44.16	39.96		

† Resistance index = emergence (pH X/pH 5.00 × 100), where X = 4.30 or 4.58.
‡ Int. = interaction; different letters in the column indicate significant entry × pH interactions at the 0.05 probability level.
§ Values in a column followed by the same capital letter are not significantly different at the 0.05 probability level by ANOVA contrast.
¶ Berseem clover mean = mean of 'Bigbee' and 'Joe Burton', red clover mean = mean of 'Arlington' and 'Cinnamon', differences within species were not significantly different at the 0.05 probability level.
# nd = not determined.
†† Range for berseem does include value for pH 4.30, where <1% of the roots emerged.

interspecific hybridization, could be a source of increased acid-soil resistance for improvement of white clover.

Results with birdsfoot trefoil in Exp. C and D indicate that birdsfoot trefoil is slightly less acid-soil resistant than white clover. Our results are in general agreement with solution-culture studies (Wheeler and Dodd, 1995a,b; Edmeades et al., 1991a,b; Schachtman and Kelman, 1991), which have usually shown birdsfoot trefoil to be less resistant to Al than white clover. Soil-based studies, however, reported that birdsfoot trefoil was at least equal to white clover in acid-soil resistance (Baligar et al., 1985), if not more resistant than white clover (Milan et al., 1990; Staley et al., 1993). This difference between solution- and soil-based results probably reflects the several soil-based factors that can affect acid-soil resistance in contrast to solution-culture studies where the effect of Al can be more completely isolated.

Within species, we found that AU Dewey was more resistant to acid-soil stress than the other birdsfoot trefoil cultivars, with the possible exception of 'Norcen' (Table 5). Norcen's intermediate RI could have been caused by improved seedling growth resulting from its relatively high seed weight (Table 1). Our conclusion about AU Dewey is in agreement with cultivar comparisons made in solution culture (Schachtman and Kelman, 1991), a soil-based, short-term very young seedling evaluation ($r = 0.88$ $P < 0.05$; Belesky et al., 1991) and soil-based, longer-term pot studies (Alison and Hoveland, 1989; Belesky et al., 1991).

Among kura clovers, 'Rhizo' and two of the other kura cultivars were more resistant to acid-soil stress than Cossack. Cossack was bred from ARS-2678, a germplasm developed at Logan, UT (R.R. Smith, personal communication, 2001). Soils from the fields where ARS-2678 was developed have a pH of ≈8.0 (D.A. Johnson, personal communication, 2001). In contrast, Rhizo was developed at Quicksand, KY, where the surface pH is ≈6.8 and subsoil pH is likely to be lower (J.C. Vandevender, personal communication, 2001). The high soil pH at Logan could explain the differences in acid-soil resistance among these cultivars.

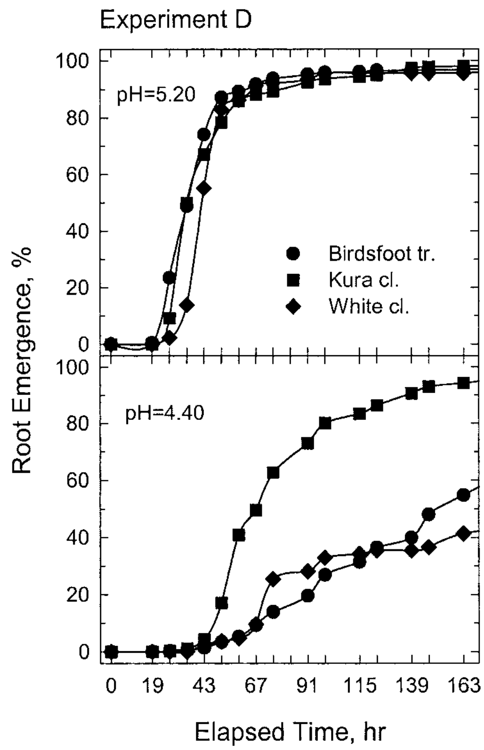

Fig. 3. Experiment D, effect of two levels of soil pH on root emergence from soil into agar across time for kura (mean of 'Cossack', 'Endure', 'KZ2', 'NF93', and 'Rhizo'), and white clover; and birdsfoot trefoil (mean of 'AU Dewey', 'Dawn', 'Fergus', 'Georgia-1', and 'Norcen').

Across all entries, seed weight was related to mean root emergence at pH 4.40 ($r = 0.89$, $P < 0.05$) and RI ($r = 0.87$, $P < 0.05$), but not to emergence at pH 5.20 ($r = -0.03$). Although we can not exclude the possibility that seed weight was responsible for part of the differ-

Table 5. Mean cumulative root emergence from soil into agar at two soil pH levels and acid-soil resistance index of clover and trefoil cultivars, Experiment D.

		Cumulative root emergence				Resistance index†
		Soil pH				Soil pH
Species	Cultivar	4.40	5.20	Range	Int.‡	4.40
		%				
Kura clover	'NF93'	56.7a§	76.2a	20	b	74.7a
	'Rhizo'	53.8ab	74.4a	21	b	72.9a
	'KZ2'	52.9ab	74.7a	22	ab	70.8a
	'Endure'	49.7ab	74.9a	25	ab	66.5ab
	'Cossack'	45.8b	78.4a	33	a	58.4b
	Mean	51.8A	75.7AB	24	B	68.7A
Birdsfoot trefoil	'Dewey'	27.6a	79.8ab	52	a	34.5a
	'Norcen'	22.2ab	84.4a	62	a	26.4ab
	'Georgia-1'	16.9b	73.9b	57	a	23.0b
	'Fergus'	15.4b	78.4ab	63	a	20.1b
	'Dawn'	14.9b	77.2ab	62	a	19.1b
	Mean	19.4B	78.8A	59	A	24.6B
White clover	'Huia'	19.3B	72.7B	53	A	26.8B
Overall mean		34.10	76.83			44.84

† Resistance index = emergence (pH 4.4/pH 5.2 × 100).
‡ Int. = interaction; different capital or lower case letters in the column indicate significant species × pH or within species (cultivar) × pH interactions, respectively, at the 0.05 probability level.
§ Values in a column followed by the same letter (lower case for cultivar and upper case for species comparisons) are not significantly different at the 0.05 probability level by ANOVA contrast.

ence in RI among these species, we believe the dramatic difference between kura clover and the other two species (Fig. 3) suggests a potentially important difference in acid-soil resistance.

CONCLUSIONS

The experiments described were run across a period of ≈1 yr. As we gained experience, minor changes were made. For example, following Exp. C, which was actually run first, we concluded that the pH value for the control treatment should be ≈5.2 so that the Al saturation levels would be <10% in the soil used. We also raised our target pH for the most severe stress treatment to pH 4.4 to reduce treatment severity and allow better root emergence of our control species, white clover. Also, we germinated large quantities of seed and sometimes began the germination of different lots at different times, based on preliminary germination tests, in order to have the best chance of having roots of the correct size for planting.

Differences in RI among cultivars can be caused by differences in root emergence at the higher or lower pH, low and high acid-soil stress, respectively, or some combination of the two. At the higher pH, 5.0 to 5.2 in these experiments, slower root emergence of one cultivar, compared with that of a second cultivar of the same species, could be caused by poor seed quality. When this occurs, differences in RI or the presence of interactions between the pH treatments are not necessarily an indication of acid-soil resistance. The problem of confounding effects of growth rate with Al resistance is not a new concern (Caradus et al., 1987). The difference we observed in RI between two alsike clovers (Table 2) could have resulted, in part, from a difference in seed quality that resulted in less growth for the cultivar with the lower RI.

Despite large differences in seed weight, our assessment of acid-soil resistance, based on root growth of very small seedlings, agrees with that reported in the literature for most species. The exception is our finding that kura is more resistant than white clover. Within species we were able to: (i) detect known differences in acid-soil resistance among birdsfoot trefoil cultivars, and (ii) detect differences among hexaploid kura clover cultivars where differences were previously unknown.

We have used the soil-on-agar procedure with even larger seed, for example, common vetch (*Vicia sativa* L.) (unpublished data, 1998). Although results were encouraging, it was clear that modifications of the technique would be necessary for seed 50 times the size of white clover.

It is difficult to make comparisons across our experiments except through reference to the white clover control that was included in all studies. Reasons for this difficulty include: (i) the soil pH levels were not identical in all studies, and (ii) times of observation were not identical for all experiments (Fig. 1–3). Genetic and environmental variation among seedlings within the cultivars can affect results, but such variablility did not preclude detection of meaningful differences among species or known differences among birdsfoot trefoil cultivars. However, the primary limitation in making comparisons across experiments is probably the soil itself. The measured soil pH, like the germplasm, is subject to sampling error as well as small errors in measurement. Also, the soil used in these experiments, although protected from precipitation, was subjected to widely varying temperatures and humidities and changed slowly with time. Thus, soil for each experiment had to be adjusted individually to obtain a pH within the desired range.

At the levels of soil pH studied in these experiments, primary root growth is sensitive to very small differences in pH. The differences between pH 5.00 and 5.24 or between 4.70 and 4.58 are clear when results for white clover are compared between Exp. B and C (Fig. 2). Root emergence parameters characteristic of a reduction in pH are: (i) a delay in emergence, (ii) a slower rate of emergence (a less steep slope), and (iii) a lower plateau of maximum emergence, if a plateau is reached. The problem associated with soil variability can be observed by comparison of the curves for white clover at the lowest pH studied (Fig. 1–3). The curves suggest a very good correspondence across Exp. A, B, and C. For Exp. D, however, initial root emergence was slightly more rapid and emergence reached a higher plateau than in the other experiments. The root emergence curve (Fig. 3, pH 4.40) suggests that the soil pH, as perceived by the white clover, was slightly higher than the measured pH of 4.40, but much less than 4.58 (Fig. 2, Exp. C, pH 4.58). This problem does not invalidate the results of Exp. D, but it does increase the difficulty of making comparisons across experiments.

Although a numerical rating system could probably be developed to characterize acid-soil resistance across these experiments, relative to Huia white clover, it is not clear that such a rating system could provide a definitive assessment of acid-soil resistance. Our results for rose clover within Exp. A (Fig. 1) illustrate some of the problems. Rose clover had a higher RI than white clover at pH 4.60, but its RI was no different from white clover at pH 4.35 (Table 2). Despite this, it was slower to 20% emergence than white clover at pH 4.60. We believe that a simple numeric system can not adequately summarize the biologically complex differences in response within an experiment, let alone provide definitive information across experiments.

Because of the sensitivity of primary root growth to changes in soil pH, caused primarily by the associated changes in available Al, the soil-on-agar technique provides an excellent assessment of acid-soil resistance of small-seeded forage-legume seedlings. The assessment based on seedlings appears closely related to results based on more mature plants. The utility of the soil-on-agar procedure, or other seedling-based techniques as selection and breeding procedures, remains to be demonstrated.

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