Subclover Early Growth Responses to Levels and Placements of Superphosphate and Ammonium Nitrate

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ABSTRACT

Single superphosophate (SSP) commonly has been used to correct soil deficiencies of P and S in rangeland seedings of annual legumes. This study evaluated rates and soil placement of P and S as SSP and N as ammonium nitrate on germination (emergence), seedling growth, and nodulation of subterranean clover (*Trifolium subterraneum* L.). The soil was a Sobrante-Las Posas association (fine-loamy or fine mixed thermic family of mollic haploxeralfs). Plants were grown from seed in three separate greenhouse experiments. The SSP levels varied from 31 to 4000 kg ha^{-1} (equivalent to 2.4 and 313 kg ha^{-1} P and 3.7 and 480 kg ha^{-1} S). Nitrogen varied from 40 to 160 kg ha^{-1}. Placements were: 5 cm below the seed, 1.25 cm side-banded, broadcast, and seed placed. Responses measured included emergence, growth rate, shoot weight, nodulation, and P toxicity symptoms. Negative effects of SSP mainly reduced emergence and increased plant toxicity symptoms and occurred at levels $\geq$1000 to 4000 kg ha^{-1}. Negative effects of N (at 160 kg ha^{-1}) were mainly on nodulation. Positive effects on most growth parameters and nodulation were largely confined to SSP levels $\leq$500 kg ha^{-1} and were best expressed by an asymptotic regression function. Placement effects generally were small except for seed-placed reduction of emergence above 1000 kg ha^{-1} SSP. Soil-banded placement treatments usually were more beneficial to growth than either broadcast or seed placed.

IMPROVEMENT of nutritional quality, forage yield and soil fertility in California's foothill annual grasslands includes the introduction of winter annual reseeding legumes. This, in turn, usually requires correction of soil nutrient deficiencies, especially of P and S (5,6,7,8). Populations of these annual legumes are self-regenerating following successful introduction and proper grazing management, and there is little similarity between the management of these annual grasslands vs. that for annual crops grown on arable land. Repeated use of mechanical tillage or seeding implements subsequent to initial establishment is limited by economic and physical (e.g., terrain) factors. In general, fertilization practices are directed to long-term stability of vegetational composition and yield (18) rather than to maximizing first-season forage yield or first-season recovery of applied nutrients.

Soils of the region are not only limiting in N, P and S but may also have a high potential for P fixation (1,10). Because adequate levels of both P and S are needed to ensure growth (12), N_2 fixation and competitive abilities of these legumes they are frequently applied together, often as single superphosphate (SSP) (17).

The use of N in legume establishment is controversial. It has been shown that the addition of low to moderate amounts of fertilizer N can be beneficial to stand establishment and early vegetative growth (2,3,9). There are N–P interactions, e.g., if N is applied at low soil levels of P and S their deficiency may be accentuated (14). If P levels are too high, detrimental influences on seedling growth can result (14,15).

Recovery of applied P ultimately depends on factors (e.g., fertilizer placement) that regulate P concentra-

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tion at the interface of the root absorptive surface with the soil solution (11,16). Dibb's review of P placement research (4) identified ten placement variations, all of which could be derived from either broadcast or band methods. Sleight et al. (16) concluded that "... localized placement of fertilizer should be more efficient than broadcast application for all fertilizers, barring adverse effects from concentrating fertilizer too near the seed."

In the annual grassland system conditions at fall germination are typically mild, with variable rainfall. Early growth occurs slowly during cool, wet winter conditions that minimize root zone salt-related injury. Integration of all factors that influence rate and placement of P and S fertilizers tends to encourage higher rates of application at at least 3- to 5-yr-time intervals and concentration of these nutrients in a small fraction of the soil volume that is immediately accessible to newly developing plant roots.

A preliminary greenhouse experiment (unpublished) and numerous field observations over a period of years had indicated that very high rates of SSP could be seed-placed with subterranean clover under typical seasonal weather conditions at germination (cool, wet) without apparent reduction in germination and seedling development. We are not aware of any published information on this aspect of stand establishment in this soil-plant-climate system.

Three hypotheses were tested in this experiment: (i), in order to minimize P fixation by soil particles and maximize P recovery over a longer time period, much higher than typically used SSP rates can be seed-placed in the initial drill establishment of subterranean clover without important reduction in germination (emergence), seedling development, and nodulation, and with minimal P toxicity; (ii) plant emergence and growth responses will be influenced by SSP placement, with soil banding being superior to either broadcast or seed-placed methods; (iii) at SSP rates resulting in P toxicity of subterranean clover it can be reduced by the concurrent addition of N.

MATERIALS AND METHODS

Two cultivars of subterranean clover (*Trifolium subterraneum* L.) Larisa and Mt. Barker, were grown from certified seed in a P-deficient (4.5 mg kg^{-1}), P-fixing, S-deficient (12) range soil. The soil was a Sobrante-Las Posas association obtained from the University of California Sierra Foothill Range Field Station, Browns Valley, Yuba County. A rototiller was used to break up the surface centimeter of soil and the surface plant litter and soil were raked off. The soil was then tilled to a depth of 10 cm, removed and passed over a 1-cm-mesh screen to remove small rocks.

The experiments were conducted in a sunlit phytotron (20) where light intensity and duration varied daily and with the seasons during which the experiments were conducted. Construction of this facility permits inside light intensity and quality to be similar to that out of doors. The temperature was held at a constant 20 °C for all experiments.

Table 1 lists the fertilizer treatments of single superphosphate (SSP) (18% P, 12% S) and ammonium nitrate (33.5% N) used in combination with four placement treatments for each of three experiments. Because SSP was in common use as a formulation to add both needed P and S, the array of treatments was based on unit weights of SSP; P and S equivalents are also given (Table 1, Fig. 1). Nitrogen was varied between 20 and 160 kg ha^{-1}, a reasonable range of values in common practice. Experiments 1 and 2 were done under winter-early spring and winter photoperiod and light intensity conditions, and Exp. 3 under midspring conditions where light conditions had seasonally improved (Table 1). Plants were grown for 32, 37, and 33 d in Exp. 1, 2, and 3, respectively. In Exp. 2 and 3, SSP levels were higher than those typically applied in order to fully assess potential toxicity, seedling growth, and nodulation responses. In Exp. 3, additional SSP levels below 125 kg ha^{-1} were used to better define the response curves at the lower P rates while retaining the higher P rates for comparability between experiments. Of the four placement treatments, broadcast is most commonly used for maintenance applications of fertilizer on rangeland (12). However, band seeding (19) or seed-placement (17) is most commonly used with initial drill seedings of introduced annual legumes.

In all experiments, the seeds were sized for uniformity and inoculated with fresh WR strain inoculant (Nitragin Co., Milwaukee, WI) using gum arabic as a binder, and pelleted with calcium carbonate. The seeds were planted in two rows

Table 1. Dates, rates of single superphosphate (SSP), P, S, and N, placement treatments, and daily solar radiation in three early growth of subclover experiments.

Treatments	Experiment 1 (27 January–28 February)	Experiment 2 (17 November–23 December)	Experiment 3 (8 March–10 April)
	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Fertilizer application†			
SSP†	0 125 250 500 1000	0 500 1000 1500 2000 2500 3000 3500 4000	0 31 62 125 250 500 1000 2000 3000 4000
P	0 9.8 19.6 39 78	0 39 78 117 156 196 235 274 313	0 2.4 4.8 9.8 19.6 39 78 156 235 313
S	0 15 30 60 120	0 60 120 180 240 300 360 420 480	0 3.7 7.4 15 30 60 120 240 360 480
N†	0 20 40 80 160		0 20 40 80 160
Placement†	5 cm below seed 1.25 cm side-banded Surface broadcast —	5 cm below seed 1.25 cm side-banded Surface broadcast In contact with seed	5 cm below seed — Surface broadcast —
KJm^{-2}‡			
Ave.	8790	7410	17860
Max	14480	11050	24230
Min.	2110	940	460
SD	3980	2980	6210
CV, %	220	248	288

† Single superphosphate, N, and placement treatments were combined factorially, with two replicates.

‡ Daily global solar radiation (WG-295) as measured at an adjacent official weather station during the three experiments.

(7 per row in Exp. 1 and 2, 10 per row in Exp. 3), 0.5 to 1.0 cm deep (to simulate drill seeding with the seed in contact with mineral soil) in 12.5-cm diam. clay pots filled with soil. All pots were individually watered to maintain adequate soil moisture. Pots were code-numbered to prevent biasing subsequent ranking of P toxicity and nodulation. Emergence percentages were determined 14 d after planting. The plants were then randomly thinned to seven per pot (Exp. 1 and 2), or to three per row (six per pot, Exp. 3). At this time, two plants in each pot (replication) were identified as the sample for all further measurements. Plants in each treatment were sampled when the average morphological development (13) of treatment replicates reached the 5.0 leaf stage. Whole plants were removed from the pots and the soil was carefully washed from the roots using a fine spray of water. The plants were then ranked for nodule size and P toxicity effects.

Nodulation was ranked as follows:

0. No nodules
1. 1 to 5 small nodules <1 mm in diameter
1.5 6 to 15 small nodules
2. ≥ 16 small nodules
3. 1 to 10 large nodules, >1 mm in diameter
4. ≥ 11 large nodules.

The P toxicity effects (Exp. 2 and 3) were ranked as follows, based on Rossiter (14,15):

1. No symptoms
2. Slight graying of leaves
3. Leaf edges brown
4. One leaf totally brown and desiccated
5. Two or more leaves totally brown and desiccated.

The shoots and roots were separated by cutting at the cotyledonary node and both were dried to constant weight (65 °C for 48 h). Shoot weights and root weights were determined from the oven-dried samples.

The experiment was a completely randomized design with two replications of a factorial array of treatments. Data were analyzed using analysis of variance. Where a significant F test was found data were further analyzed using LSD (0.05) comparisons for the placement treatments within each experiment and linear and nonlinear regressions were used to fit the P and N level data.

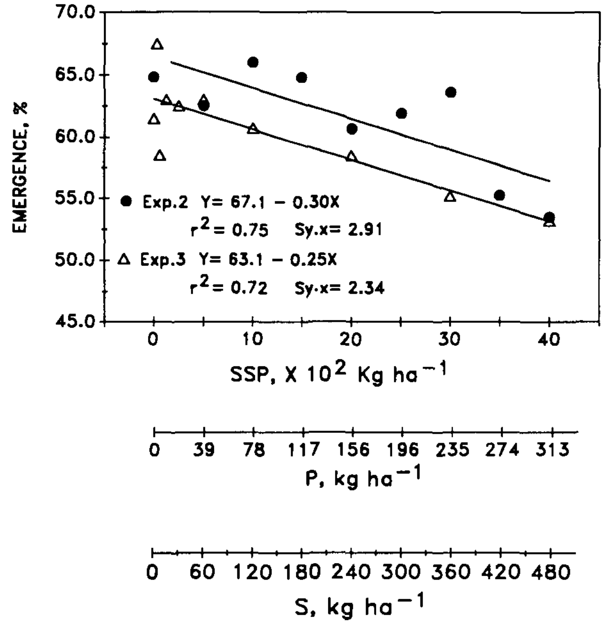

Fig. 1. Percent emergence of subterranean clover grown at increasing levels of single superphosphate.

RESULTS AND DISCUSSION

Single Superphosphate and Nitrogen Rate Effects

Emergence. There were no differences ($P > 0.05$) in percent emergence values over the range of SSP or N levels in Exp. 1 (data not included). A negative linear trend was observed in Exp. 2 and 3 (Fig. 1), largely because of the influence of SSP levels $\geq$ 2500 kg ha^{-1}. A linear regression calculated from data from 0 to 2500 kg ha^{-1} had virtually no slope ($63.4 - 0.087X$, $r^2 = 0.08$) while the equation using data from 2500 to 5000 kg ha^{-1} was $73.9 - 0.498X$, $r^2 = 0.73$. The only significant reduction in emergence due to N was from the 160 kg ha^{-1} level in Exp. 3; these data are not reported.

Shoot Weights. Shoot weight responses to SSP were limited to levels below 60 kg ha^{-1} S and 39 kg ha^{-1} (500 kg ha^{-1} SSP) (Fig. 2). At higher SSP rates shoot weights neither increased nor decreased and the three data sets were well fitted to an asymptotic regression function of the form

$$Y = A\,(1 - be^{-CX}). \quad [1]$$

Root weights varied little and data are not reported. Calculated shoot/root ratios for all three experiments also followed Eq. [1] (average $R^2 = 0.957$).

Nodulation. Addition of SSP to 1000 kg ha^{-1} improved nodulation with little additional effect above that level (Fig. 3a). The data could be combined for all three experiments and adequately expressed by the use of Eq. [1] ($R^2 = 0.91$). While nodulation was improved to 500 to 1000 kg ha^{-1}, of equal importance is the fact that it was not depressed at much higher SSP rates.

Conversely, as expected, an increase in N levels decreased nodulation (Fig. 3b). In both experiments where responses to levels of N were evaluated (Exp. 1, 3) nodulation rank declined to similar values at the highest N rate. The initial decline apparently was steeper in Exp. 1, possibly because of the lower light levels at that time of year (Table 1), and hence represented an energy partitioning response. As with nodulation responses to SSP rates, data from the two N rate experiments could be combined ($R^2 = 0.90$) and were well fitted to an equation of the form

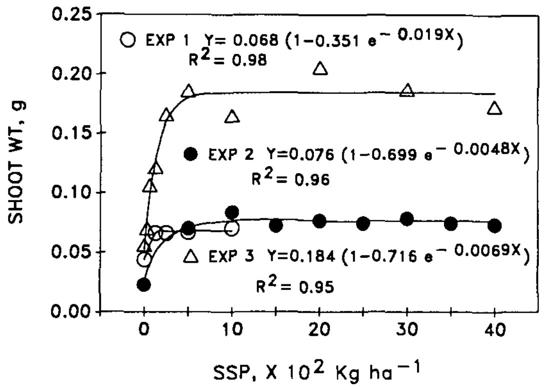

Fig. 2. Shoot weight at the five-leaf stage of subterranean clover grown at several levels of single superphosphate.

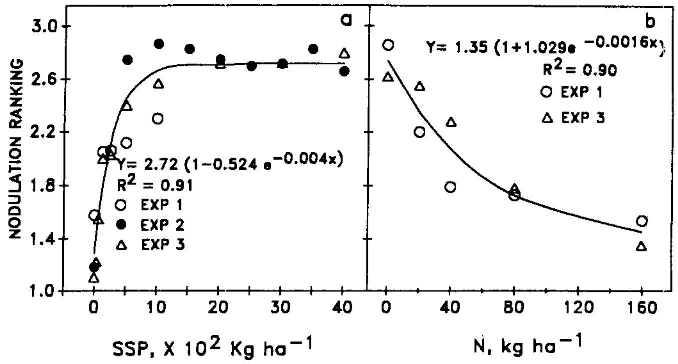

Fig. 3. Nodulation responses to: a) increasing levels of single superphosphate and b) N.

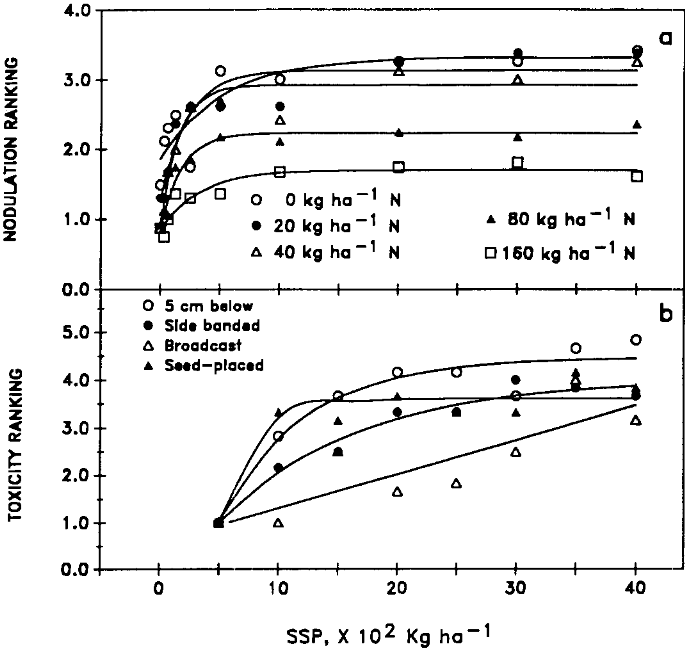

Fig. 4. Interactions of increasing levels of single superphosphate to: a) nodulation response at five N levels, and b) toxicity responses at four placement treatments.

$$Y = A\,(1 + be^{-cx})\,. \qquad [2]$$

An interaction between SSP and N occurred where the N treatment responses formed three groups (Fig. 4a, Table 2). At 2000 kg ha^{-1} SSP and above, nodulation at 20 or 40 kg ha^{-1} did not differ from control, while both 80 and 160 N levels gave successively lower nodulation rankings. Despite these obvious differences in asymptotic values, the pattern of asymptotic response at increasing SSP rates was reinforced, as the average R^2 value for the five N treatments was 0.89 (Table 2). The clustering of asymptote values for the 0, 20, and 40 kg ha^{-1} N treatments suggested that where P and S are nonlimiting N depression of nodulation at low to moderate N levels may not occur.

Other Considerations. In order to fully assess the potential of emerged seedlings for continued normal growth at very high SSP rates, certain other plant growth and development parameters also were measured. These included leaf appearance (growth) rate and leaf area per plant at sampling. There was little evidence that growth rate was depressed at the higher SSP levels. Data from all three experiments also followed the asymptotic regression function (Eq. [1]). The R^2 average was 0.97 and the major difference between experiments was in the asymptote (3.6–5.0). This variation could be explained by seasonal differences in light (and, possibly, surface soil temperature) environments.

Table 2. Regression equations and R^2/r^2 values for interactions of N vs. single superphosphate (SSP) on nodulation (Exp. 3, Fig. 4a) and for interactions of placement vs. SSP on toxicity ranking (Exp. 2, Fig. 4b).

	Nodulation ranking	
N level	Regression	R^2/r^2
kg ha^{-1}		
0	$3.31(1-0.436e^{-0.0019X})$	$R^2 = 0.77$
20	$3.13(1-0.595e^{-0.0045X})$	$R^2 = 0.89$
40	$2.92(1-0.711e^{-0.0068X})$	$R^2 = 0.94$
80	$2.24(1-0.619e^{-0.0057X})$	$R^2 = 0.96$
160	$1.71(1-0.505e^{-0.0031X})$	$R^2 = 0.89$
	Toxicity ranking	
Location	Regression	R^2/r^2
5 cm below	$4.47(1-1.55e^{-0.0014X})$	$R^2 = 0.93$
Side-banded	$4.01(1-1.16e^{-0.00087X})$	$R^2 = 0.96$
Broadcast	$0.584 + 0.072X$	$r^2 = 0.71$
Seed-placed	$3.61(1-4.71e^{-0.0038X})$	$R^2 = 0.89$

Responses where levels of fertilizer similar to those used in this experiment are applied to naturally regenerating annual plant communities of mixed species composition may be different because of competition effects from associated plants. This factor was not assessed in these experiments.

Phosphorous Toxicity Effects

Using Rossiter's (14,15) criteria for evaluating P toxicity, SSP effects were demonstrated (Fig. 4b, Table 2). We found little evidence of an N reversal of P toxicity in these experiments and data are not reported. Values at the 20, 80, and 160 N levels for 2000, 3000, and 4000 kg ha^{-1} SSP were not different from control while values for 40 N were significantly greater ($P < 0.05$) than the control. These data appear to suggest that N may have increased P toxicity at 40 N while decreasing it at 80 and 160 N. This inference is tenuous and would require further research to document.

Placement × SSP rate interactions for toxicity rank also were variable (Fig. 4b, Table 2), with the greatest variation in toxicity rank associated with the broadcast treatment ($r^2 = 0.71$, Table 2). However, toxicity rank values were numerically lowest for the broadcast treatment, at least to 3000 kg ha^{-1} SSP. It appeared that toxicity response increased most rapidly for seed-placed or banded-below-seed treatments as SSP rates were increased (Fig. 4b). Considering the excellent fit of data from the two seed-placed treatments (average $R^2 = 0.93$, Table 2) the data imply additional interaction as SSP rates increase, and a tendency for toxicity rankings to converge at some common maximum value.

Table 3. The effect of fertilizer placement on emergence, shoot weight, and root weight of seedling subterranean clover plants.

	Fertilizer placement treatment			
	5 cm below the seed	Side-banded 1.5 cm from the seed	Broadcast	Seed placed
		Emergence (%)		
Exp. 1†	79a‡	73b	82a	
Exp. 2	69a	64b	71a	38c
Exp. 3	68a		53b	
		Shoot weight (g)		
Exp. 1	0.03a	0.04b	0.04b	
Exp. 2	0.08a	0.07a	0.07a	0.06b
Exp. 3	0.15a		0.14a	
		Root weight (g)		
Exp. 1	0.03a	0.04b	0.04b	
Exp. 2	0.03a	0.03a	0.03a	0.02a
Exp. 3	0.10a		0.09b	

† Experiments defined in Table 1.

‡ Values having the same letter within an experiment (rows) are not significantly different ($P > 0.05$).

Placement Effects

Placement influences were variable (Table 3). The seed-placed treatment reduced emergence to 38% from 71% in the broadcast treatment and emergence in the side-banded treatment was lower than when fertilizer was placed 5 cm below the seed ($P < 0.05$). A placement × SSP interaction was found for emergence, where the seed-placed treatment caused a significant ($P < 0.05$) reduction in emergence with SSP levels $\geq$1000 kg ha^{-1}. Placement effects on shoot and root weights were minor although shoot weight was decreased ($P < 0.05$) by the seed-placed treatment (Table 3). Greater differences occurred between experiments than between treatments within an experiment.

Nodulation generally was numerically better when SSP was soil banded or broadcast than when seed-placed, but in general placement effects were small.

CONCLUSIONS

Plant responses to a wide range of SSP and N treatments were consistent over three experiments conducted at different times of the normal growing season.

Addition of 500 to 1000 kg ha^{-1} SSP to this soil corrected deficiencies of P and S and improved rate of leaf appearance, shoot weight, leaf area per plant, shoot/root ratio, cotyledon area and vigor, and nodulation, but levels of SSP at or above 2500 kg ha^{-1} tended to decrease percent emergence.

Although most of these favorable responses occurred at SSP levels $\leq$500 kg ha^{-1} ($\leq$39 kg ha^{-1} P, 60 kg ha^{-1} S), they also were not diminished at SSP levels much higher than required for initial (stand establishment) correction of soil P and S deficiencies.

Plant P toxicity symptoms showed a consistent increase at SSP levels $\geq$1000 kg ha^{-1} and P toxicity was not clearly reduced by addition of N. At the levels employed in this experiment, N had generally minor influences except for decreasing nodulation.

Overall, placement effects were minor except for the seed placed treatment at SSP levels over 1000 kg ha^{-1}, but if soil and other conditions permit, placement of SSP or similar supplementary fertilizers below the seed or as a side-band at initial seedings are preferable over broadcast or seed placed applications.

Despite the observed limitations, application levels of SSP that can be economically justified can be applied in legume stand establishment in annual rangelands using available placement methods without unduly jeopardizing legume seedling emergence and initial stand establishment.

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