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THE ESTABLISHMENT OF RED CLOVER ON ACID SOILS AS INFLUENCED BY LIMING, SEED COATING, AND SELECTED *RHIZOBIUM TRIFOLII* STRAINS

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ABSTRACT

A field trial was conducted to determine the influence of liming, seed coating, and strain of acid-tolerant *Rhizobium trifolii* on establishment and first-year production of red clover (*Trifolium pratense* L.) on acid soils. The experiment was conducted during 2 years, each year on a new soil site. The initial pH of the experimental sites was 5.3. Lime, added at rates of 5 or 10 metric tons/ha, produced soil pH values of 5.9 and 6.3, respectively, in soil samples collected at a depth of 0-15 cm. Six *R. trifolii* strains and three seed-coating treatments were used. Seed was uncoated or coated with an amended lime admixture using the 'Prillcote' coating process. Parameters measured included stand count, nodulation, plant height, dry matter yield, and nitrogen content of the herbage. Red clover plant population at establishment was enhanced by seed coating but was not influenced by the strain of rhizobia added to the seed. Dry matter yields were increased the second year by liming acid soils and seed coating but were not affected by rhizobia strains used. The optimum lime:seed coating ratio varied between years. Significant differences in plant height attributable to seed coating were noted during the second year of the study. Lime application and seed coating were both important factors influencing red clover production in the year of seeding.

INTRODUCTION

The establishment and production of red clover (*Trifolium pratense* L.) in the North Central United States is frequently limited as a result of soil-related problems. Among these are poor soil fertility and pH. Lime coating of seed and selection of more acid-tolerant strains of *Rhizobium* spp. may improve stands and yields of forage legumes produced on acid sites.

In an early study of alfalfa (*Medicago sativa* L.) nodulation, Karraker (1927) grew alfalfa plants in pots with soils varying in pH by depths. He reported a reduction in nodule numbers in the acidic portion of the soil. Also, in the acidic soil a total nodule count of 8 was obtained, whereas, in the limed soil a total count of 104 was determined.

Lowther and Loneragan (1968) reported that root infection on nodule initiation had a higher calcium (Ca) requirement than either nodule devlopment or host plant growth in the presence of fixed nitrogen (N). Munns (1969) found that production of pectinase precedes infection. Pectinase activity was inhibited by acidity in the pH range of 4.5 to 5.5, which coincided with the pH range that has been shown to be inhibitory to root hair infection. He indicated that the pH optimum for pectinase activity may determine the pH at which nodulation will take place.

John et al. (1972) showed in a growth chamber experiment that increased yields and a decreased growth period to reach harvest stage were the beneficial effects of lime application and could be attributed to reduction in the concentration of aluminum (Al) and manganese (Mn). Munns et al. (1977) stated that increased nodulation and plant N content agreed with the conclusion that, for most species, growth improvement associated with liming was due to improved N fixation.

Increasing the inoculation rate per seed markedly enhanced the growth of white clover (*Trifolium repens* L.) and could replace the need to use lime-coated seed on some moderately acid soils (Lowther, 1974; Lowther, 1975; Lowther and Loneragan, 1968). Baker et al. (1968) noted that, when soil moisture was adequate during establishment, maximum alfalfa yields were obtained with lime pelleting when soil acidity levels were below pH 5.9.

According to Date (1970), the inoculant rhizobial population must develop rapidly. Low rhizobial numbers can mean delays in nodule initiation with subsequent growth retardation and even stand loss. Chatel and Greenwood (1973) concluded from their studies that the ability of *R. trifolii* to colonize a host and become established in soil varied with the strain tested.

The interrelationships of liming, seed pelleting, and introducing selected *Rhizobium* spp. to field soil have not been investigated thoroughly under midwestern United States climatic and soil conditions. Previous studies have not examined the effects of lime application on the plant growth response to seed coating and the microbial response to seed coating and lime application. The objectives of this study were to determine the influence of seed coating, selected *R. trifolii* strains, and rates of lime application on establishment and seeding year production of red clover when grown on acid soils.

MATERIALS AND METHODS

This 2-year study was conducted at the Cooperative Agronomy Research Center, Southern Illinois University, Carbondale, IL, on two adjacent sites which had been in permanent sod. A new site was used each year to prevent changes in the indigenous rhizobial population from exerting an influence on the second year's treatments. The soil on both experimental sites was a Stoy, fine-silty, mixed mesic, Aquic Hapludalf. The sites were fall plowed to incorporate lime at the rate of 0, 5, and 10 metric tons/ha. Soil samples collected after application of lime had pH values of 5.3, 5.9, and 6.3 (0 to 15 cm deep) for the 0, 5, and 10 metric ton/ha lime treatments, respectively. Levels of soluble Mn in the soil were very high (90 to 127 ppm), soluble Al levels were low (3 to 19 ppm), and Ca and molybdenum (Mo) levels were moderate to high (800 to 1500 ppm and 0.1 to 0.3 ppm, respectively) at all lime rates.

In 1978 a factorial design was used, and in 1979 a split-plot design was used. Four replications of each seed treatment were used both years. Plot size was 1 m by 5 m. A broadcast application of 112 kg/ha P_2O_5 and 224 kg/ha K_2O was made prior to final seedbed preparation. Before planting, the herbicide EPTC (S-ethyl dipropylthiocarbamate) was applied to the soil at the rate of 0.7 liters/ha a.i. and incorporated.

Red clover (cv. 'Kenland') seeds from the same lot with a germination of 96% were coated with an amended lime admixture (0.56 g thiram, 1% MgO, 1% K_2SO_4, Ohio peat inoculant plus peat per 200 g seed) and inoculated with *R. trifolii*. These seeds were planted with a 'Planet Jr.' seeder on May 1, 1978, and May 23, 1979, in rows 20 cm apart. The *R. trifolii* used in this study were provided by CelPril Industries, Inc. and were selected on the basis of acid soil tolerance from a wide geographical area. For comparison, a commercial inoculant was used for one treatment. The strains, inoculation rate, and seed-coating mixture used in this study are listed in Table 1. Seeding rates were 5.4, 8.3, and 10.8 kg/ha for red clover and coating ratios of 0:1, 0.5:1, and 1:1 (kg of lime:kg of seed), respectively. Irrigation was applied as necessary.

Stand density was measured 3 and 6 weeks after planting by counting the number of plants per m^2. Plant height was used as an index of seedling vigor and was measured 6 weeks after planting. Root nodulation was rated 6 weeks after sowing and 3 weeks after the first harvest. Nodulation ratings were done visually. Ratings were based on the number of plants out of five selected at random from each plot which exhibited effective nodulation (pink nodules). Dry matter yield determinations were made at the half bloom stage by harvesting 4 m^2 from each plot. Dried plant samples were ground to pass a 2-mm screen and analyzed for nitrogen content using the Kjeldahl method. Data for each year were evaluated by analysis of variance. Significant means were separated by using orthogonal contrasts at the 5% level of significance.

RESULTS AND DISCUSSION

Seed coating improved stands at 3 and 6 weeks over the control in both 1978 and 1979, regardless of lime rate (Table 2). The differences were highly significant. In 1978, there was no difference at 3 weeks in stands where seed coat ratio of 0.5:1 and 1:1 had been used. However, by 6 weeks a small, but significant, difference was evident between the seed coatings, with plots seeded with the 1:1 coating ratio having the better stand. In 1979, the stand from the 0.5:1 seed coating was significantly higher at both 3 and 6 weeks. The plant counts generally increased with higher seeding rates.

Liming did not influence plant populations except at 6 weeks in 1978 when the 10 metric ton/ha lime rate produced denser stands than the 5 and 0 metric ton/ha lime rates (Pr F = 0.0102). A lime by seed coating interaction was not significant. Scott (1975) stated that seed coating influences stand development in the first 3 weeks of plant development with its influence becoming less recognizable as seedling development continues and subsequent germination of non-coated seeds takes place. From our results, it appears that seed coating influenced stand development to at least 6 weeks.

The decrease in plant population between 3 and 6 weeks in 1978 was due to insufficient rainfall which caused many of the seedlings to die. Irrigation was applied after the 6-week measurements were made. In 1979, dry weather conditions prevailed again and supplemental water was applied soon after planting. Dowling et al. (1971) reported that lime-coated seed imbibed at a fast initial rate with non-limiting water, resulting in higher germination of coated seed. It is quite likely that the presence of adequate soil moisture during germination contributed to the increased number of plants observed with the coated seed than with the raw seed. There were fewer red clover plants m^2 in 1979 than 1978 at 3 weeks. This was attributable to soil crusting in 1979 which limited seedling emergence.

Seed treatment, seed coating, and *R. trifolii* strain selection failed to produce any discernible differences in the number of plants nodulated either 6 weeks after planting or 3 weeks after the first harvest. The non-inoculated seed treatments, (Table 1), showed average nodulation. This result suggests that the indigenous rhizobial population responded to the introduction of red clover to the site by multiplying, infecting, and nodulating the clover roots. Furthermore, effects of the *R. trifolii* pelleted onto the seed may have been masked by the build-up of the indigenous rhizobial population and were undetectable by our methods. When 10 metric/tons/ha were applied in 1978, liming significantly increased nodulation above the control 6 weeks after planting (Pr $F = 0.0061$) and 3 weeks after the first harvest (Pr $F = 0.0304$) (Table 3). The first harvest was made about 70 days after planting and the second harvest about 50 days later. Again, the decrease in nodulation between sampling dates in 1978 was likely due to moisture stress.

Red clover plant height was not affected by liming in either year of the study. Seed coating significantly increased plant height in 1979 (Pr $F = 0.009$) over all lime rates. The 0.5:1 coating produced plants that were taller than the other treatments. A lime by seed coating interaction was not significant.

Due to a weed infestation after the first harvest in 1978, only one red clover cutting was obtained, however, two harvests were made in 1979. There was no significant difference among first harvest yields in 1978 and 1979 due to lime application. The second red clover harvest showed liming increased dry matter yields (Pr $F = 0.002$). This suggests that the yield response of red clover to applied lime is delayed. Both the 10 and 5 metric ton/ha lime rates produced more total dry matter than the control in 1979 (Table 4).

Seed coating increased first and second harvest dry matter yields in 1979 (Table 5); however, the increase for the 1:1 coated seed was not significant in the first harvest. The increased yields due to seed coating can be attributed to greater plant numbers as well as to increased height of the plants. The effects of seed coating and lime application appear to complement one another; the seed coating influences seedling establishment and liming influences stand productivity from the first harvest onward. Again, the lime rate by seed coating interaction was not significant.

Herbage N content was not enhanced by any strain of *R. trifolii* used in this study or by liming as Munns (1977) suggested. Likewise, no difference for other parameters measured could be attributed to strain of *R. trifolii* used. This suggests that the inoculant strains introduced were unable to compete with or were no more effective than the indigenous population of *R. trifolii* that were present in the soil. Chatel and Greenwood (1973) also concluded that the ability of *R. trifolii* to colonize a host and become established in soil varies with the strain tested.

In conclusion, seed coating was shown to increase red clover stands, regardless of lime application, in both years of the study. Increase in plant population and plant height in response to seed pelleting produced higher dry matter yields than the non-coated seed in the second year of the study. The response of red clover to added lime appeared to be delayed, becoming evident in the regrowth the first harvest. The results suggest that seed coating and lime amendments complement each other and improve the seasonal productivity of red clover stands. Liming also tended to increase the number of plants nodulated, but not consistently. No evidence was found that any of the *R. trifolii* strains used were superior to the indigenous rhizobial species in their abiltiy to produce nodulation and fix atmospheric nitrogen.

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Table 1. Red clover seed treatments for 1978 and 1979.

Treatment	Inoculant Strain	Number of Rhizobia X 10^6/seed	Seed Coat Ratio +
			kg of lime: kg of seed
1	Nitragin "AB"	‡	not coated
2	2751	50	0.5:1
3	2751	50	1:1
4	27631	50	0.5:1
5	27631	50	1:1
6	2233	50	0.5:1
7	2233	50	1:1
8	2764OP	50	0.5:1
9	2764OP	50	1:1
10	2207	50	0.5:1
11	2207	50	1:1
12	none	none	0.5:1
13	none	none	1:1
14	2751	50 +	0.5:1

+ All treatments except #1 contain the following: 0.56 G. Thiram, 1% Mg0, 1% K_2SO_4, Ohio peat inoculant plus Peat per 200 G of seed.
‡Number of rhizobia/seed were not determined for this treatment. The treatment was not included in 1979.

Table 2. The effect of lime coating red clover seed on stands at 3 and 6 weeks.

Seed Coat Ratio (kg of lime: kg of seed)	3 weeks	6 weeks
1978 (Planted May 1)	Plants/m^2	
0:1	177	96
0.5:1	236	124
1.0:1	255	144
Contrast 0 vs 0.5 and 1.0	**	**
1979 (Planted May 23)		
0:1	98	75
0.5:1	153	157
1.0:1	109	118
Contrast 0 vs 0.5 and 1.0	**	**
Contrast 0.5 vs 1.0	**	**

**Significant at the 5 and 1% level, respectively.

Table 3. Mean root nodulation of red clover 6 weeks after planting 1 May 1978 and 28 May 1979 and 3 weeks after the first harvest as influenced by lime.

Lime	Nodulation Rating‡			
	6 weeks after planting		3 weeks after first harvest	
(Metric ton/ha)	1978	1979	1978	1979
0	1.8	3.1	0.55	4.7
5	3.5	3.3	0.64	4.9
10	4.0	3.5	1.07	4.9
Contrast 0 vs 5 and 10	**	NS	**	**
Contrast 5 vs 10	NS	NS	**	NS

‡Ratings were based on visual evaluation of 5 plants randomly selected from each plot. The rating scale ranged from 0 (poor nodulation) to 5 (excellent nodulation).
**NS. Significant at the 1% level, and not significant respectively.

Table 4. Total seasonal dry matter yield of red clover as influenced by lime application during 1978 and 1979.

Lime Rates	Dry Matter Yields (Kg/ha)	
(Metric tons/ha)	1978 (One harvest)	1979 (Two harvests)
0	1,423	4,592
5	1,423	5,371
10	1,498	6,012
Contrast 0 vs 5 and 10	NS	**
Contrast 5 vs 10	NS	**

**NS. Significant at the 1% level, and not significant respectively.

Table 5. Effect of seed coating on dry matter yields of red clover, 1979.

Seed Coating Ratio (kg of lime: kg of seed)	First harvest (Aug. 1)	Second harvest (Sept. 20)
	----------kg/ha----------	
0:1	1,114	3,059
0.5:1	1,693	3,971
1.0:1	1,355	3,824
Contrast 0 vs 0.5 and 1.0	*	**
Contrast 0.5 vs 1.0	**	NS

*, **, NS. Significant at the 5 and 1% level, and not significant, respectively.