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Population Size and Effectiveness of Clover Rhizobia From Fields in the Vicinity of Corsicana

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Summary

Soils from the Corsicana region were collected in order to determine if clover inoculation is necessary every year. Soil pH and rhizobial population were determined for each soil and dry weights of plants inoculated with the soils were measured. Variations in soil pH, rhizobial population sizes, and effectiveness of the rhizobia were observed.

Introduction

Crimson clover and arrowleaf clover are common forage legumes in east Texas pastures. One of the advantages of growing legumes is the reduced need for fertilizer nitrogen, since legumes in association with the root nodule bacteria *Rhizobium* are capable of fixing atmospheric nitrogen. Because arrowleaf and crimson clovers are introduced species in Texas the appropriate rhizobia are not naturally present. It is necessary to inoculate with the appropriate strains of *Rhizobium* the first time the legume is planted in order to achieve optimum levels of N_2-fixation. Annual clovers may become re-established in the fall as a result of natural reseeding. In such cases it is not a common practice to provide a commercial inoculation, but instead, rely on established rhizobial populations in the soil for nodulation of clovers. The ability of clover rhizobia to survive through the summer in Texas has not been determined. The objective of this study was to determine the population size of rhizobia in fields that have grown clover and relate it to soil pH and frequency of growing clover.

Materials and Methods

Soils were collected in October 1988, prior to the planting of clover for the 1988 season. Twenty-one pastures in the Corsicana area were sampled. Soil pH was measured using a 1:1 soil:water ratio. The population of *R. leguminosarum* bv. *trifolii* in each soil was estimated by the most probable number method using arrowleaf clover grown in growth pouches (Weaver and Frederick 1982). Effectiveness of the population of rhizobia in each soil was determined by measuring plant dry weights of both Yuchi arrowleaf and Dixie crimson clover inoculated with 0.1 g of soil and grown in growth pouches in a growth chamber for 21 days. A population was rated effective if the plant dry weight was 50 percent greater than that of the uninoculated control plants. A second experiment was conducted in a greenhouse using the same plant species grown in 1 L pots filled with vermiculite. Eight soil samples having high rhizobial populations and four with low rhizobial populations were selected as inoculants. Ten grams of the appropriate soil was added as inoculum to the pots at the time of planting. A randomized complete block design with two replicates was used. Effectiveness of the rhizobial populations was determined as in the first experiment by measuring plant dry weights after 42 days growth.

Results and Discussion

At the time of sampling clover plants were not present. The population of rhizobia in each soil was expected to represent survival from the end of the growing season in early July to the next growing season in late October. It was assumed that the population would be high at the beginning of the summer immediately following senescense of the clover.

Rhizobial population size ranged from 0 to 3×10^5 cells g^{-1} soil (Table 1). Population size did not appear to be related to the number of years of clover production (Table 1). Sites 1, 2, and 3 had been in production for 15 years with inoculant added with reseeding each of those years but only one site (1b) contained a large population of rhizobia. In comparison, site 15 had a large population of clover rhizobia even though it had been in clover production for only 2 years. Three sites (11, 17, and 20) were planted to clover in the previous year and had large rhizobial numbers in one sample of each soil but the other samples contained low rhizobial populations. In the two sites never planted to clover (13 and 21), rhizobial populations were not detected.

The number of years since the last commercial inoculation treatment did not have a consistent effect on the rhizobial

lower population of rhizobia, as illustrated by site 1. Most of the soils with pH above 5.0 had comparatively large populations. Again, there are exceptions such as site 19b which had a pH of 7.8 and a population of 200 cells g^{-1} soil.

As determined from the dry weight of the plants grown in growth pouches only five sites (4, 7, 9, 12, and 20) had rhizobial populations effective with arrowleaf clover and eight sites (4, 7, 9, 12, 14, 15, 17, and 20) had rhizobial populations effective with crimson clover (Table 1). All of these soils had large rhizobial populations. However, not all soils with large populations had effective rhizobial populations (Table 1).

Effectiveness of the rhizobial populations on both crimson and arrowleaf clovers for selected sites was further tested in a longer term experiment in the greenhouse. The soils indicating ineffective rhizobial populations in the first experiment (8, 13, 18, and 21) showed similar ineffectiveness in the greenhouse experiment since the plant dry weights of either arrowleaf or crimson clover were not different from the uninoculated treatment. Soils with effective rhizobial populations on both arrowleaf and crimson clovers in the first experiment (4, 9, 12, and 20) demonstrated effectiveness in the greenhouse experiment also. Among the other three soils with effective rhizobial populations, site 17 had rhizobial populations effective only on crimson clover in the first experiment but was effective on both plant species in the greenhouse. Sites 14 and 16 were ineffective on both species in the first experiment but effective in the greenhouse test. Differences in the effectiveness ratings between the two experiments are probably related to the short growth period in the first experiment or due to the difference in the quantity of inoculum utilized.

The soils studied represented a wide range of variability in pH, texture, moisture regime, vegetative cover, management and rhizobial population size, and effectiveness. In several instances rhizobial population size in samples from the same field were dissimilar (sites 1, 9, 11, 17, and 20; Table 1). Because of these variabilities in the populations and poor correlation with the measured parameters, it is not possible to predict the surviving rhizobial population size. Additional research is needed to develop an understanding of factors that influence the population size and the critical population size requiring the use of a commercial inoculant to ensure good nodulation.

Literature Cited

1. Weaver, R. W. and L. R. Frederick. 1982. *Rhizobium. In*: Methods of soil Analysis, Part 2. Chemical and Microbiological Properties (A. L. Page, R. H. Miller, and D. R. Keeney Eds). pp. 1043-1070. American Society of Agronomy, Madison, WI.

population size (Table 1). Sites 1, 2, and 3 had been repeatedly inoculated for 15 years but still had low rhizobial populations. Site 14 was recently inoculated and contained a high population of rhizobia while site 4 was last inoculated with a commercial inoculant 8 years prior to sampling and contained a large population of rhizobia.

The pH of the soils varied from 4.2 to 8.2 (Table 1). Variation in the rhizobial population size did not relate directly to the soil pH. Seven of the sites had a pH below 5.0 and four of these sites had very low populations. Of the other three acidic sites, a large degree of variability existed between the replications. Usually the soil sample had the lower pH and a

TABLE 1. POPULATION SIZE AND EFFECTIVENESS[1] OF CLOVER RHIZOBIA IN 21 PASTURES AND THE INFLUENCE OF YEARS OF GROWING CLOVER, INOCULATION, AND SOIL PH

Site	Years of clover	YSLI[2]	Soil pH	Rhizobia	Growth chamber Dry Weight AC	CC	Greenhouse Dry Weight AC	CC
				No.g^{-1}soil	mg plant^{-1}		mg plant-1	
1a*	15	1	4.5	66	5	28	-	-
1b			4.9	130,000	6	27	-	-
2a	15	1	4.5	0	6	25	-	-
2b			4.5	17	6	23	-	-
3a	15	1	4.4	40	7	21	-	-
3b			4.7	7	7	21	-	-
4a	9	8	6.8	130,000	14	43	40	89
4b			6.7	69,000	5	24	-	-
5a	3	3	5.8	100	5	19	-	-
5b			5.5	2,300	5	16	-	-
6a	5	1	6.7	10,000	6	26	-	-
7a	5	1	5.1	7,000	11	28	-	-
8a	10	4	4.2	7	5	23	-	-
8b			4.9	130	5	21	9	14
9a	3	3	7.6	46,000	8	42	23	102
9b			7.7	210	6	21	-	-
10a	5	5	4.2	680	6	23	-	-
10b			4.8	2,500	5	24	-	-
11a	1	1	6.2	2,400	5	26	-	-
11b			5.1	30	5	26	-	-
12a	3	3	7.5	140,000	11	51	36	93
12b			7.5	29,000	10	33	40	87
13a	N**	N	4.6	6	6	22	-	-
13b			5.6	0	5	18	9	16
14a	3	1	4.6	7,100	6	31	-	-
14b			5.8	13,000	7	21	32	56
15a	2	2	6.3	14,000	4	32	-	-
15b			5.5	14,000	7	14	-	-
16a	3	N	6.2	2,600	6	25	35	95
16b			4.6	400	6	21	-	-
17a	1	1	5.5	1,700	7	34	34	72
17b			5.1	12	5	22	-	-
18a	6	6	6.4	0	5	20	8	12
18b			6.7	0	6	22	-	-
19a	3	3	7.9	2,000	4	22	-	-
19b			7.8	200	6	18	-	-
20a	1	1	8.0	35	5	29	-	-
20b			8.2	310,000	9	36	37	102
21a	N	N	8.0	0	5	19	6	18
21b			7.9	0	5	19	-	-
control			-		5	20	6	13

[1] As indicated by plant dry weight of inoculated arrowleaf clover(AC) and crimson clover (CC).
[2] Years since last inoculation.
* a and b represent two samples from the same site.
** Never.