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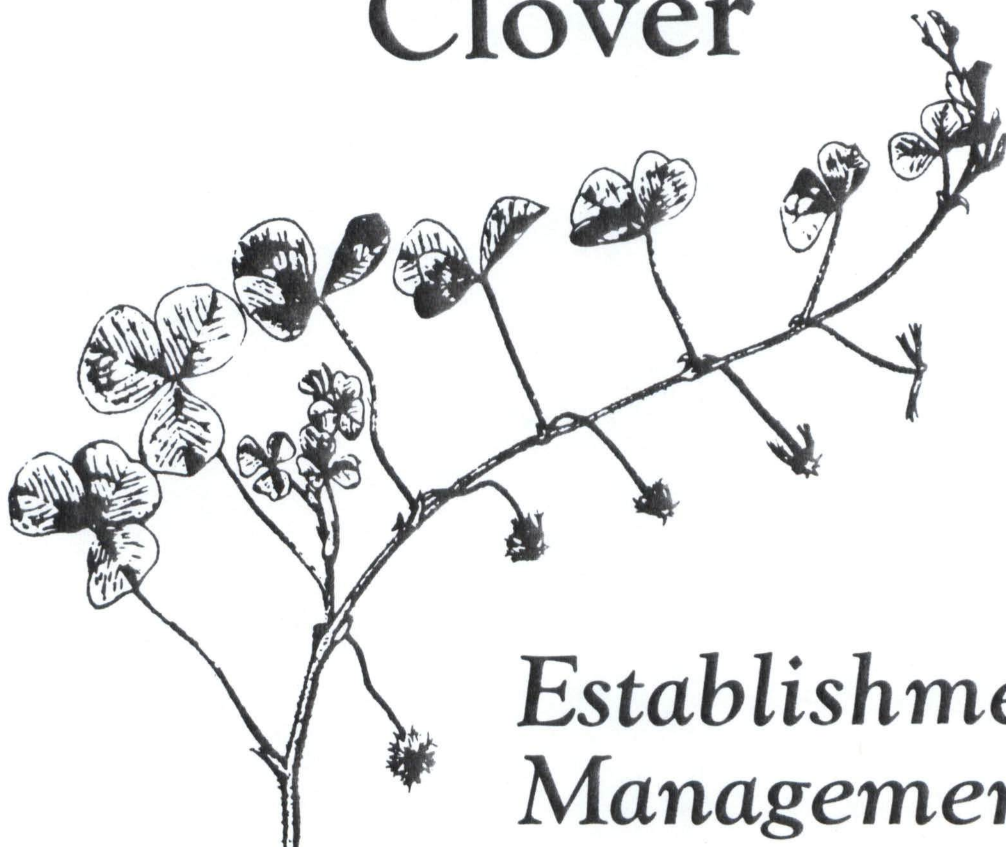
Subterranean Clover



Establishment, Management, and Utilization in Texas

THE TEXAS AGRICULTURAL EXPERIMENT STATION/Neville P. Clarke, Director/The Texas A&M University System/College Station, Texas

CHAPTER 3

Clover Inoculation

R. W. WEAVER

Nearly all of the chapters acknowledge the importance of nitrogen fixation as one of the advantages of growing subclover. Nitrogen fixation can only occur if the plant is nodulated by the appropriate bacteria. These bacteria, called rhizobia, are able to penetrate the cell wall of root hairs with the aid of enzymes (10, 5) and move through the root hairs into the cortical cells of the plant root (7). Rhizobia are much smaller than the root hairs, which are not individually distinguishable by the unaided eye. If these rod shaped rhizobia are placed end to end it takes approximately five to reach across the diameter of the root hair which is a distance of approximately five ten-thousandths of an inch. The bacteria stimulate the plant to produce hormones that result in the formation of nodule tissue. The nodule is comprised of numerous plant cells which become filled with rhizobia (10). Inside the nodule, the rhizobia produce an enzyme called nitrogenase that converts atmospheric nitrogen gas into ammonia, a form of nitrogen that plants can utilize.

Two genera of rhizobia exist and are recognized by their growth rates (11) and their ability to nodulate particular host plants. Within each genus there are many different strains that may be characterized on the basis of their ability to form nodules on particular hosts (2), fix nitrogen (effectiveness), survive unfavorable environmental conditions, and on their antigenic constituents. The particular type of rhizobia that is effective on a leguminous plant may not naturally be present in a soil. The first time a legume is planted in a field it is very important to provide the appropriate rhizobia as a seed inoculant so that the plant will rapidly nodulate and receive the maximal benefit from nitrogen fixation (18, 19). The yield of clovers planted for the first time in Texas soils is often doubled by inoculation (Table 1).

Rhizobia are living organisms that have particular nutritional and environmental needs for survival and others for multiplication. The nutritional and environmental constraints for survival are not nearly as restrictive as the constraints for reproduction. Reproduction requires the development of new cells but survival only requires maintenance of existing cells. Rhizobia do not form spores and generally can only survive temperatures of 120°F (15) for a short while. Multiplication of cells is maximal at temperatures of approximately 80°F (2) and will continue to approximately 100°F after which multiplication will cease and weaker cells will die.

Availability of water is critical to survival of rhizobia (4, 15, 17). Rhizobia do not survive well when undergoing moisture stress and must be stored in some material that retains moisture. Inoculant carriers provide the necessary protection against desiccation and an easy means for handling the rhizobia. Most often the carrier is peat which is packaged in a plastic bag and sealed to prevent moisture loss (2). The peat provides a good substrate for multiplication of the rhizobia and after the maximal population is reached, the peat provides a good nutritional and physical environment for an adequate shelf life of the product.

Rhizobia exposed to sunlight and desiccation following surface sowing of seed die-off rapidly (9). Soil provides some protection against desiccation, but as a soil dries out the rhizobal population decreases. Rhizobia isolated from nodules of clovers grown in Texas displayed differential ability to survive in a dry soil (Table 2). When the soil was moist all of the isolates survived quite well, but when the soil was too dry for seeds to germinate, only some strains were able to maintain satisfactory population levels.

Clover seed does not provide a hospitable environment for survival of rhizobia (13, 14). Clover seed coats contain substances that are toxic to the rhizobia and other substances that may interfere with the iron uptake by the bacteria (6). Survival is very dependent on the storage conditions following inoculation of the seed (21). Storage at normal temperatures that occur during the fall planting season will result in the death of most of the rhizobia within 24-48 hours after inoculation (Table 3). To extend the survival of rhizobia, a coating material (Table 1) should be used to adhere the inoculant to the seed. Most commercial adhesives contain gum arabic or methyl cellulose which are very good and superior to homemade preparations (2). A solution of sucrose, table sugar, may be used as a substitute (1), but it is not as good a product as the materials provided by the inoculant companies and may attract insects to the seed.

After inoculation, the seed should be planted as soon as possible and delaying more than 1 day should be avoided. The seed may survive when sown onto a dry soil but most of the rhizobia will die while waiting for the seed to germinate (15, 9). The result will be poor nodulation (16) and reduced seedling vigor. The seed should be sown into the soil to provide protection to the rhizobia against dessication (Table 4).

High populations of rhizobia, in the range of one thousand per seed, will result in nodules being apparent on the root system by the three leaf stage, and the nodules will be located on the upper portion of the root (Tables 1 and 4). The sites of infection occur in the root hair region which is located near the tip of growing roots. Any portion of the root is only susceptible to infection and nodule formation for a few hours and naturally, the upper portion of the root is the first to develop and have infection sites. A low population of rhizobia has to multiply before infection and by the time this multiplication occurs the early sites for infection are no longer available. It is very important that infection occur as early as possible so that the nodules can form and begin to provide the plant with nitrogen.

Table 1. Effect of Inoculant Adhesive and Rate of Inoculation on Nodulation and Dry Matter Production of White Clover the First Time It Was Planted in a Field

Adhesive	Rate	No. Nodules on Taproot	Yield
	No. Rhizobia per seed	per plant	lbs/acre
Uninoculated	—	5a*	1,420a
Water	600	9ab	1,220a
Gum Arabic	600	19c	2,060b
Gum Arabic	3,000	15cb	2,300b

*Means, within columns having letters in common are not significantly different at the 5% level according to Duncan's Multiple Range Test.

Table 2. Differences in Survival of Clover Rhizobia in a Sterilized Soil Incubated at 90°F for Two Days at Two Moisture Levels

Rhizobia isolate	Soil Moisture Level %	
	2*	4
	No. per g soil	
1	10	63,000
2	18	10,000
7	9,000	100,000
11	10,000	600,000

*This moisture level was too low for seed to germinate. All populations were present at 1 million per g at the beginning of the incubation.

Table 3. Survival of Rhizobia on Clover Seed as Influenced by Time After Inoculation and Relative Humidity When Stored at 86°F

Time	Relative Humidity	Survival
	Percent	
0	75	100
0	100	100
24 hours	75	1.6
24	100	10
48	75	0.17
48	100	2.6

Table 4. Effects of Planting Depth and Inoculation Adhesive on Numbers of Rhizobia Surviving Two Days and Number of Nodules on the Taproot of Arrowleaf Clover After Thirty-five Days

Adhesive	Planting depth	No. Rhizobia surviving	Nodule number
		per seed	per plant
Uninoculated	3/8″	—	0.1a
Water	surface	<20	1.0ab
Water	3/8″	<20	—
Gum Arabic	surface	100	3.8c
Gum Arabic	3/8″	3,000	10.8d

Many rhizobia are able to form nodules on the plant but this does not insure that nitrogen will be provided to the plant (3). Fortunately the specificity is not so large for subterranean clover as occurs for some other clovers. Inoculant companies provide a product for each clover type and their recommendations should be followed.

Because subclover is adapted to acid soils (12), there may be some concern for survival of the rhizobia. It has been my experience that survival of rhizobia in soils of pH 5.0 and above is not limited by pH. Limited research results in Texas indicate that growth of subclover on soils more acidic than pH 5.0 is very poor. If more acidic soils prove to be satisfactory for clover growth, it may be important to lime the soil to enhance survival of the rhizobia or coat the seed with finely ground limestone at planting to provide a favorable micro-environment for rhizobial survival (20).

When planting subclover in Texas, special measures need to be undertaken when inoculating and planting to enhance survival of the rhizobia. Seeds should be sown during the time of year when they will promptly germinate and become established. The population of rhizobia declines rapidly in soil that does not provide for prompt germination of seed. Use an inoculant sticker to extend the survival of the rhizobia, to provide for uniform coating of the seed for good distribution of the inoculant, and to keep the inoculant near the seed in the soil. If feasible, plant the seed into the soil as opposed to surface sowing in order to extend the survival of the bacteria. Future research needs to be conducted to evaluate the benefits of other methods of inoculation such as adding the inoculant directly into the soil (8), developing seed coating materials to protect the rhizobia from toxic seed coat factors, and isolating or genetically engineering highly effective strains of rhizobia that are resistant to drought stress.

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