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COMPARISON OF INOCULATION TREATMENTS FOR ROSE CLOVER

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Summary

Seedling growth of rose clover (*Trifolium hirtum* All.) is slower than other annual clover species. The poor seedling performance may be due to limited N_2-fixation by present inoculation procedures. HiStick inoculant was compared to Pelinoc and an uninoculated control on 'Overton R18' rose clover seedlings in the greenhouse. Both inoculation treatments produced more nodules per seedling and on the top 1.2 in. of the tap root than the control at 40 days. By 53 days, both inoculation treatments still had more nodules in the top 1.2 in. of the tap root than the control. Leaf number, nodule number and seedling weight were never significantly different between the two inoculation treatments. However, seedling weight was always highest in the HiStick treatment, followed by Pelinoc and then the control. HiStick inoculant was equal to, but not better than, Pelinoc inoculation on rose clover.

Introduction

Overton R18 is the first rose clover variety selected for the southeastern US (Smith et al., 1992). It matures 3 to 4 weeks later and is twice as productive as other available varieties. Poor seedling growth and slow nodulation restricts early forage production of rose clover (Evers, 1993). In the rhizobia-plant symbiotic relationship, the rhizobia provide nitrogen (N) to the plant for growth and the plant provides energy in the form of carbohydrates to the rhizobia. The amount of N fixed by rhizobia for the plant is determined by rhizobia strain, supply of carbohydrates provided by the plant, soil N level, and environmental factors. It is not known if the poor seedling growth of rose clover is limited by

moderately effective inoculation methods which limit N to the plant or if the slow seedling growth is characteristic of the species.

Improved inoculation techniques have increased nodulation and seedling growth of arrowleaf clover (*Trifolium vesiculosum* Savi.) (Evers et al., 1993). New inoculation procedures which enhance rhizobia survival and/or new rhibozia strains with improved N_2-fixation ability may improve rose clover seedling growth. A greenhouse study was conducted at the Texas A&M University Agricultural Research and Extension Center at Overton to evaluate HiStick inoculant

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which contains a new rhizobia strain on rose clover.

Procedure

Potting material consisted of 25% peat moss and 75% sand. After mixing, the potting material was placed in a soil sterilizer at 180°F for 5 hr. Plastic, 6-in. pots were washed, rinsed in 5% bleach solution, triple rinsed and allowed to dry. Seventy-two pots were each filled with 4.3 lb of soil and moistened with 3 oz (100 ml) of distilled water containing 70 g KH_2PO_4/5.3 gal of water. Scarified Overton R18 rose clover seed was sterilized in a 20% bleach solution which was placed on a magnetic stirrer for 5 min. After removing from the bleach solution, the seed was triple rinsed in distilled water and then soaked in distilled water for 6 hr to enhance germination. After drying, the seed was divided into three equal lots that were either, 1) inoculated with Pelinoc, 2) inoculated with HiStick, or 3) uninoculated control.

Fifteen seeds were planted per pot on 26 May 1994 and thinned to 10 seedlings after emergence. Pots were watered approximately every other day alternating between 3 oz of distilled water or KH_2PO_4 solution. Seedlings were watered once with Peter's Soluble Trace Element Mix (1 teaspoon per 5 gal water) after emergence. Temperature controls for the greenhouse were set for 60°F nights and 75°F days. Experimental design was a randomized complete block with four replications. Two pots from each inoculation treatment in each replication were removed at 25, 40, and 53 days after planting. Rose clover seedlings were carefully removed from the pots and washed to remove potting mix. Number of fully expanded leaves and nodules per seedling were recorded. Nodule number in the top 1.2 in. (3 cm) of the tap root was also recorded at the 40 and 53 day sampling. Seedlings were dried in a forced air oven at 140°F for 48 hr and weighed to determine dry weight per seedling. Standard analysis of variance was performed on the data with mean separation by Waller-Duncan Multiple Range Test at 0.05 level.

Results and Discussion

There were no significant differences between treatments at the first sampling date 25 days after planting (Table 1). Although every effort was made to sterilize seed and potting material, nodulation still occurred on seedlings in the uninoculated treatment. At the second sampling date, clover seedlings in the control treatment had significantly fewer nodules per seedling and in the top 1.2 in. of the tap root (Table 2). Nodules in the upper part of the tap root is an indication of early nodulation. Infection by *Rhizobium* bacteria occur on root hairs near new root growth. Studies with other annual clover species indicate that nodulation sites remain susceptible to

rhizobia infection for only one to two days (Nazih and Weaver, 1994). In excess of 1000 rhizobia per seed are required for good nodulation. Lack of nodules in the upper part of the tap root on the control seedlings indicate nodulation occurred about 1 wk after germination. Many of the nodules found on the control seedlings were small which indicates infection by ineffective rhizobia strains.

By the third sampling date 53 days after planting, there were significant differences only for leaf number and nodules in the top 1.2 in. of the tap root (Table 3). Number of nodules per seedling were similar for all treatments, but many of the nodules on seedlings from the control treatment were small. Differences in seedling weight were never statistically different. However, seedlings from the HiStick inoculation treatment were always the largest followed by Pelinoc and then the control treatment.

Data from this study show that HiStick was equal to Pelinoc for inoculation of rose clover. Significant differences between the uninoculated and inoculation treatments did not always occur due to nodulation of the uninoculated seedlings. Although every effort was made to keep the greenhouse cool, the long, hot summer days probably limited rose clover growth. Therefore, differences between treatments were not as great as they might have been under more favorable autumn climatic conditions.

Literature Cited

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Table 1. Influence of inoculation treatment on leaf number, nodule number, and dry weight per rose clover seedling 25 days after planting.

Treatment	Leaf no.	Nodule no.	Weight
			g
Control	2.47	6.08	0.030
HiStick	2.70	6.48	0.033
Pelinoc	2.38	5.40	0.032

Table 2. Influence of inoculation treatment on leaf number, nodule number, nodules on upper 1.2 in. of tap root, and dry weight per rose clover seeding 40 days after planting.

Treatment	Leaf no.	Nodule no.	Nodules top 1.2 in.	Weight
				g
Control	4.95	6.63 b[1]	0.85 b	0.076
HiStick	5.30	11.69 a	2.88 a	0.086
Pelinoc	4.90	12.85 a	3.00 a	0.080

[1]Values within a column followed by the same letter are not significantly different at 0.05 level. Waller-Duncan Multiple Range Test.

Table 3. Influence of inoculation treatment on leaf number, nodule number, nodules on upper 1.2 in. of tap root, and dry weight per rose clover seedling 53 days after planting.

Treatment	Leaf no.	Nodule no.	Nodules top 1.2	Weight
				g
Control	7.75 b[1]	20.55	1.60 b	0.120
HiStick	8.35 a	23.90	3.33 a	0.144
Pelinoc	7.85 ab	20.60	4.33 a	0.126

[1]Values within a column followed by the same letter are not significantly different at 0.05 level. Waller-Duncan Multiple Range Test.