Liquid Inoculant for Chickpea

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Abstract

Three *Rhizobium* sp bv *Cicer* strains were formulated in a liquid carrier and evaluated for their ability to nodulate desi chickpea in the greenhouse. The population of the strains was monitored over **5** months, the maximum time anticipated between manufacture and the farmer applying it to seed. All strains were applied to seed at a rate that provided a population that exceeded 1X1 0^5 cells per seed. Only the plants that were inoculated developed nodules and all strains were effective forming pink-red nodules on the crown and lateral roots. Shoot fresh weight of inoculated plants was always greater than that of the uninoculated plants. The population of each strain remained at or above $1X10^9$ cells per mL after 5 months storage at 4°C.

Introduction

Chickpeas have generated much interest within the farming community in the last two years. This crop is well suited to the great plains of Canada because of its drought tolerance, nitrogen fixing capability that benefits crop rotation, adaptability to current cultural practice in the prairies and upright stand of the crop for ease of handling at harvest time.

Chickpea is a legume and therefore is capable of fixing its own nitrogen following a successful association with an effective strain of *Rhizobium.* All nitrogen-fixing crops benefit from inoculation of nitrogen-fixing bacteria. This is particularly true of crops that are being introduced into a new area. However, on land that has a history of legume production ie. pea and lentil, a yield benefit was observed when the crop was inoculated (Hynes et al. 1995).

Agrium Biologicals has had a long and productive research history in biological nitrogen fixation and legume production in western Canada. In 1991 the first liquid *Rhizobium* inoculants for peas and lentils were introduced by Esso Ag Biologicals (now Agrium Biologicals). In 1993 a new liquid bean inoculant was developed. following extensive screening for a strain of *Rhizobium* that was capable of nodulating bean that had been treated with captan and streptomycin. The recent interest in chickpea production and forecast of acres for this crop easily warranted the development of a chickpea inoculant for western Canada.

The goal of this research was to select a superior strain of *Rhizobium* from greenhouse and laboratory studies and evaluate its performance in the field. Packaging and shelf-life of the inoculant were also evaluated.

Materials and Methods

Microorganisms

Three strains of *Rhizobium* sp bv *Cicer* that had been previously characterized by LiphaTech as effective strains on chickpea were prepared in a liquid formulation in Milwaukee, WI. The strains were identified as 27A2, 27A7, 27A9.

Shelf-life studies

The viable counts of *Rhizobium* sp in the liquid formulation were determined periodically by removing samples by syringe through a silicon septum on the bags, serially diluting the sample and spreading aliquots of the diluent onto yeast extract-mannitol agar (Nelson and Child, 1981). Colony-forming units were counted after 3 day's incubation at 27°C.

Greenhouse studies

Chickpea variety 90R-01 was obtained from Bert Vandenberg of the Crop Development Centre. Seed (50g) was treated (0.2 mL) with one, two and four month-old inoculum and planted within 2 hours in sterilized modified Leonard jar assemblies (Vincent, 1970) containing a mixture of turface and perlite (2:1) and provided with Hoagland's (Hoagland and Boyer,1936) N-free nutrient solution. Triplicate treatments included seed treated with each of the strains in the liquid formulation and an uninoculated control. After 6 weeks in the greenhouse the plants were rated for colour and hardiness and then removed and the roots were gently washed. The crown and lateral root nodules were counted and weighed. This was replicated 3 times and analysis of variance was carried out using Fishers Protected LSD.

Results

The *Rhizobium* strains were prepared in a liquid formulation by a commercial manufacture and packaged into bags similar to those that we use to distribute our lentil, pea and bean inoculant. The population of each strain after five months in storage is shown in figure 1. These results indicate that the population of the three strains was stable in this liquid formulation. Four month-old inoculant nodulated the plants as well as 1 month-old inoculant indicating that the inoculant maintained not only a high population but that the *Rhizobium* remained highly effective.

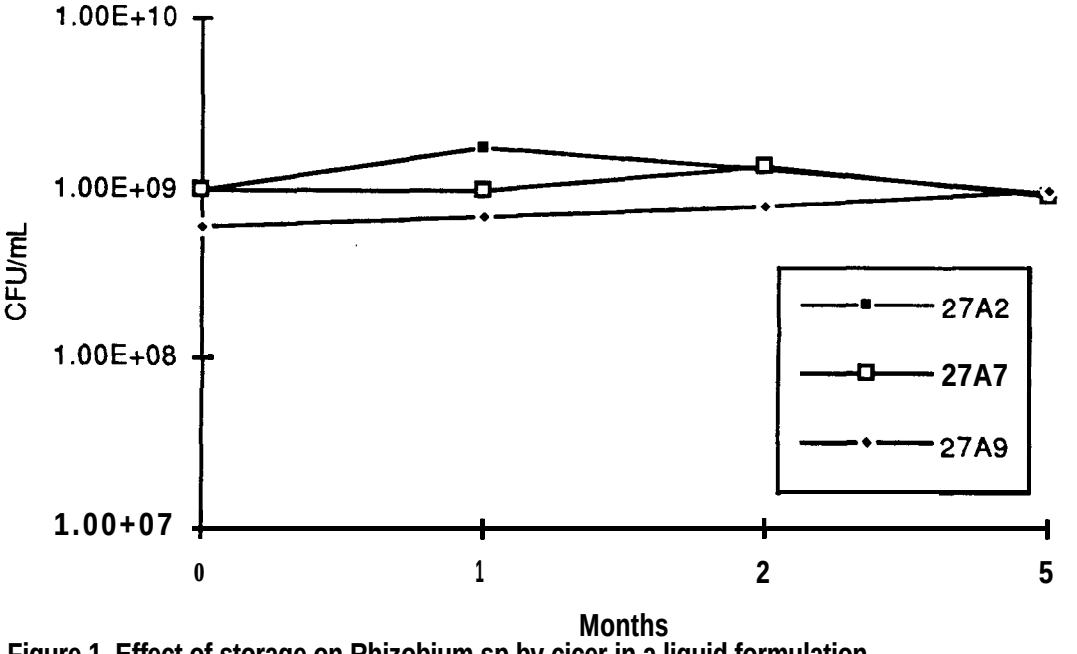

Figure 1. Effect of storage on Rhizobium sp bv cicer in a liquid formulation.

After six week's growth in the greenhouse visual differences between the inoculated and uninoculated plants were easily detected. The leaves of the uninoculated plants were pale green to yellow-green, indicative of nitrogen limitation, whereas the leaves of the inoculated were dark green. Fresh shoot weight, nodule number and mass measurements are shown in table 1. The uninoculated plants did not accumulate as much shoot mass as the inoculated plants and as expected there were no nodules. Nodule number and mass were not significantly different among the strains tested (Table 1). With regard to nodule number per plant, *Rhizobium 27A2* was the most consistent strain and was considered to be the best performer.

Table 1. Effect of liquid inoculant on shoot mass, nodule mass and nodule number of desi chickpea 90R-01 inoculated with *Rhizobium* sp **bv** *cicer.*

Treatment	Fresh Shoot Mass(g)	Nodule Number	Nodule Mass(g)
control	2.54	0	0
27A2	2.95	24'	0.36*
27A7	2.98	27'	0.36*
27A9	3.63*	22*	0.32*

***Significant at the 95% confidence level using Fisher's Protected LSD.**

Discussion

Crop rotation on the prairies has traditionally been used to prevent the development of high populations of disease causing microorganisms in the soil and change the weed profile. Crop rotations with legumes promote the above benefits and provide slow release nitrogen for the subsequent crop. Wheat quality following a pea crop frequently is improved and the protein level may be 1 percent or higher. These benefits can only be past on to a subsequent crop if the legume was inoculated becomes well nodulated.

Inoculation of the seed with an effective nitrogen-fixing *Rhizobium* and providing adequate fertility are the key steps towards a successful legume crop. *Rhizobium* strain selection under the environmental conditions that exist on the Canadian prairies has been used by this group for the selection and development of our inoculants for lentil (Bremer et al. 1990), field pea (de Jong et al. 1986, 1987 and 1988, Hynes et al. 1995) and dry bean.

Laboratory and greenhouse studies have suggested that *Rhizobium* sp *cicer* 27A2 was the superior strain on desi chickpea. Field studies in Saskatchewan are planned for the summer of 1996 to examine the effectiveness of this strain.

Acknowledgements

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