

# LIQUID INOCULANT FOR DRY BEANS

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All of the beans seeded in western Canada are treated with the fungicide captan and while it is effective against soil-borne root diseases it has been reported to inhibit symbiotic nitrogen fixation by *Rhizobium*. Therefore, we set out to isolate a strain of bean rhizobia that could nodulate and fix nitrogen in the presence of captan. Several strains of *Rhizobium leguminosarum* bv *phaseoli* were screened for tolerance to captan. One strain was advanced into greenhouse studies and inoculated onto pinto CV Othello seed treated with and without captan. Greenhouse studies showed that the captan tolerant strain of *Rhizobium* nodulated captan treated plants as well as plants not treated with captan. Field trials with inoculated captan-treated and untreated seed confirmed the results observed in the greenhouse. This strain of *Rhizobium* is commercially prepared in a liquid formulation and is available for farmers under the trade name "RhizUp™ for Beans".

## Introduction

Inoculating seed with rhizobia ensures that a large and effective population is available in the rhizosphere of the young plant. Symbiotic N<sub>2</sub> fixation occurs following the association of effective rhizobia with its host plant. Up to 60% of the plant's N requirement is made available following the conversion of atmospheric N<sub>2</sub> by the rhizobia (Rennie, 1986; de Jong, 1987; van Kessel and Nelson, 1989).

In 1991 the first liquid *Rhizobium* inoculants, sold by Imperial Oil under the trade names Enfix P and Enfix L for field pea and lentil, respectively, was commercially available for western Canadian farmers. These liquid formulations provided farmers with a user friendly inoculate that is faster, easier to use, much less messy than the traditional peat powders. Following the successful introduction of these inoculants it became apparent that the dry bean producers desired a liquid inoculate. All dry bean seeded in western Canada are treated with captan, a fungicide that has been reported to inhibit nodulation by *Rhizobium* (Rennie 1986).

We obtained several strains of *Rhizobium leguminosarum* bv *phaseoli* and screened them for tolerance to captan for the purpose of developing a liquid inoculant for the dry bean producers.

## Materials and Methods

### Strains

Four strains of *Rhizobium leguminosarum* bv *phaseoli* were obtained from the Agriculture Canada, Lethbridge AB. culture collection. The strains were designated *Rhizobium leguminosarum* bv *phaseoli* as 1 through 4.

### Laboratory and greenhouse studies

Each strain was grown in the liquid formulation at 22°C on a shaker operating at 175 rpm. After 3 days of growth the *Rhizobium* was examined for tolerance to captan by spreading 100µL of each of the cultures onto yeast-mannitol agar containing captan.

Strains of *Rhizobium* that grew on solid media in the presence of captan were used to inoculate captan-treated pinto variety Othello bean seed (Alberta Bean Pool) and pinto variety Othello seed not treated with captan. Seed not inoculated with *Rhizobium* was also included as controls. Seed was planted into soil collected from Raymore SK. that had been blended with vermiculite (medium grade) and sand (3:2: 1) and placed into 6" pots. The pots were placed into the greenhouse and watered daily. Three times per week the plants were watered with Hoagland's N-free nutrient solution (Hoagland and Boyer, 1936). Nodule number and nitrogen fixation ( $C_2H_2$  reduction) were assessed 4 to 5 weeks after planting.  $N_2$  fixation ( $C_2H_2$  reduction) was measured by removing the plants, usually mid morning, separating the roots from the shoots followed by a gentle washing of the roots with water and placing them into 500 mL screw-cap jars fitted with a rubber seal. Acetylene was added to a final concentration of 5%. After 30 min. gas samples (5 mL) were removed and stored in vacu-tainers in field studies or analysed immediately. Gas samples were analyzed for  $C_2H_2$  and  $C_2H_4$  using a Poropak R column fitted into a Shimadzu gas chromatograph GC14A equipped with a flame ionization detector operated at 200°C and  $N_2$  carrier gas at 30 ml/min. Roots were dried for 48 hours at 70°C and weighed.

### Field Studies

*R. leguminosarum* bv *phaseoli* was prepared in the liquid formulation at LiplaTech Inc., Milwaukee. The liquid formulations were packaged into plastic bags containing 4 L of inoculum to treat approximately 1089 kg of seed or 40 bu. Farmers selected by the Alberta Bean Pool were asked to test the inoculum. The inoculum was spread over the seed in planter box of their seeder just prior to seeding. Nodulation and nitrogen fixation ( $C_2H_2$  reduction) was assessed 5 weeks after planting.

## **Results**

### Laboratory and greenhouse studies

Only one of the 4 strains of *R. leguminosarum* bv *phaseoli* was capable of growth on YMA plus captan. This confirmed the results of Rennie (1986). Captan-treated and non-chemical treated pinto bean cv Othello had 3 and 34 nodules per plant, mean of nine plants. Plants not treated with inoculum had less than ten nodules per plant (Figure 1). Nodules developed in the crown and lateral regions of the root and were red when cut open indicating that they were actively fixing nitrogen. Nitrogen fixation ( $C_2H_2$  reduction) of captan-treated and non chemical-treated seed were similar (Figure 2) and about 5 times greater than the uninoculated controls.

**Figure 1.** Enfix B Nodule number on captan-treated plants.

**Figure 2.** Nitrogen fixation activity on captan-treated seedlings.

{--- Figures at end of paper ---}

## Field Studies

Field experiments were carried out at the farms of F. Koch and K. Nishi, Bow Island, AB. Plants were gently dug from the rows and the soil was washed from the roots. Acetylene reduction was assessed as described in the materials and methods. Inoculated plants had become nodulated in the crown region of the root whereas the uninoculated plants had very few nodules in the crown region and some nodules on the lateral roots. Acetylene reduction was 2 to 3 times greater with inoculated plants (Figure 3).

**Figure 3.** Nitrogen fixation activity of bean root nodules

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## **Discussion**

Establishing an effective **N<sub>2</sub>-fixing** symbiosis is highly dependent on the carrier to provide a large and effective population of rhizobia for the host plant. We have demonstrated that a liquid inoculant for lentils and field peas is as effective as the traditional peat inoculant (Hynes et al. 1995). The bean producers of western Canada had also requested a liquid inoculant, however, bean seed is treated with captan, a fungicide known to inhibit nitrogen fixation by *R. leguminosarum* bv *phaseoli* (Rennie 1986). The mode of action of captan on *Rhizobium* is not known at this time. Following a screening procedure in the laboratory and testing of the strain in the greenhouse a strain of *R. leguminosarum* bv *phaseoli* has been found that nodulates captan treated pinto bean seed.

Farmers are beginning to recognize the benefit of growing legumes in rotation with other crops but many have excluded legumes because of the difficulties of inoculation. The ease of application of a liquid inoculant may encourage more farmers to diversify and include legumes in their planting schedule.

## **Acknowledgments**

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Enfix B Nodule number on captan-treated plants

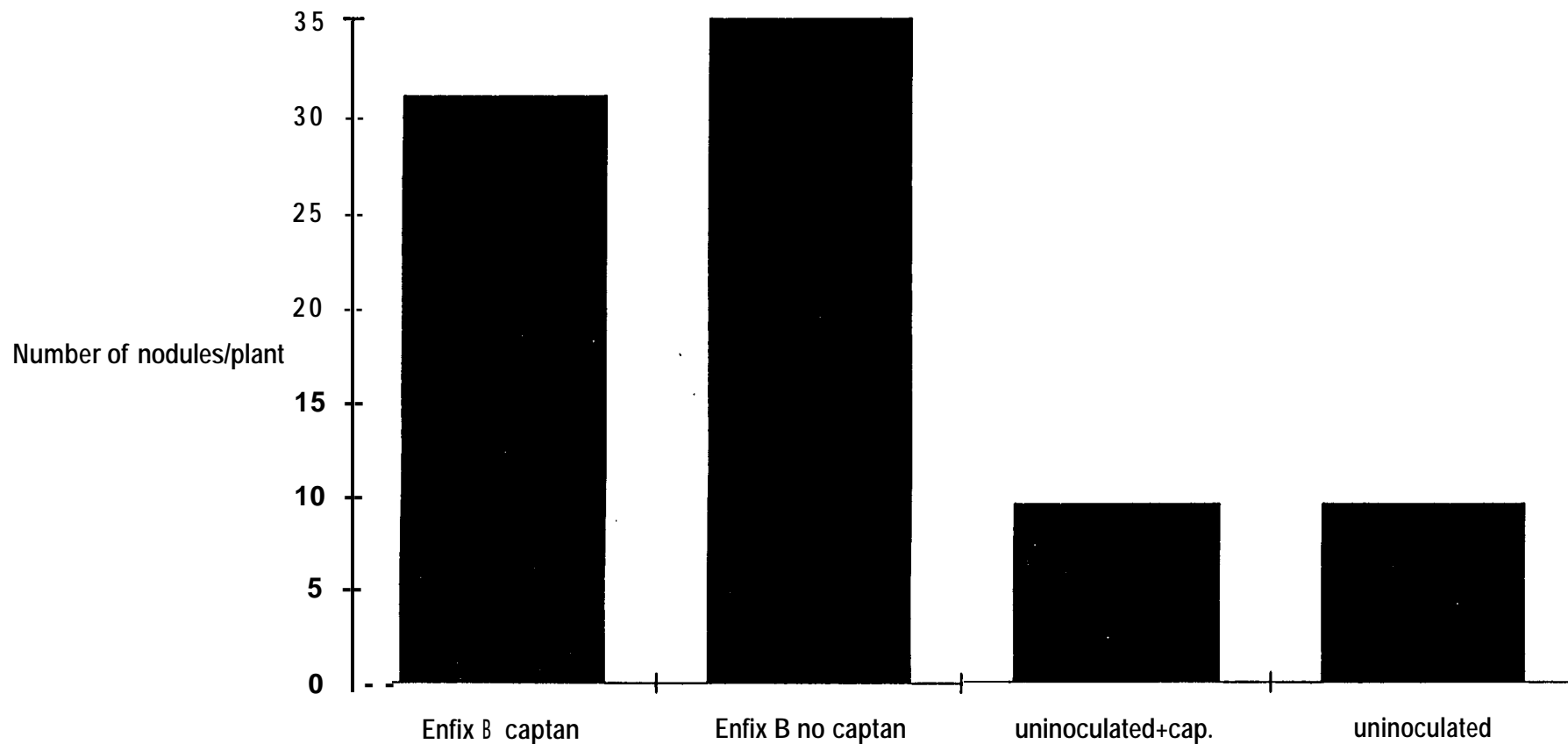


Figure 1.

**Nodule Rating:** Captan-treated and non captan-treated plants had nodules on the tap root. Nodules from both treatments were red when cut open indicating that they were fixing nitrogen.

### Enfix B Nitrogen fixation activity on captan-treated seedlings

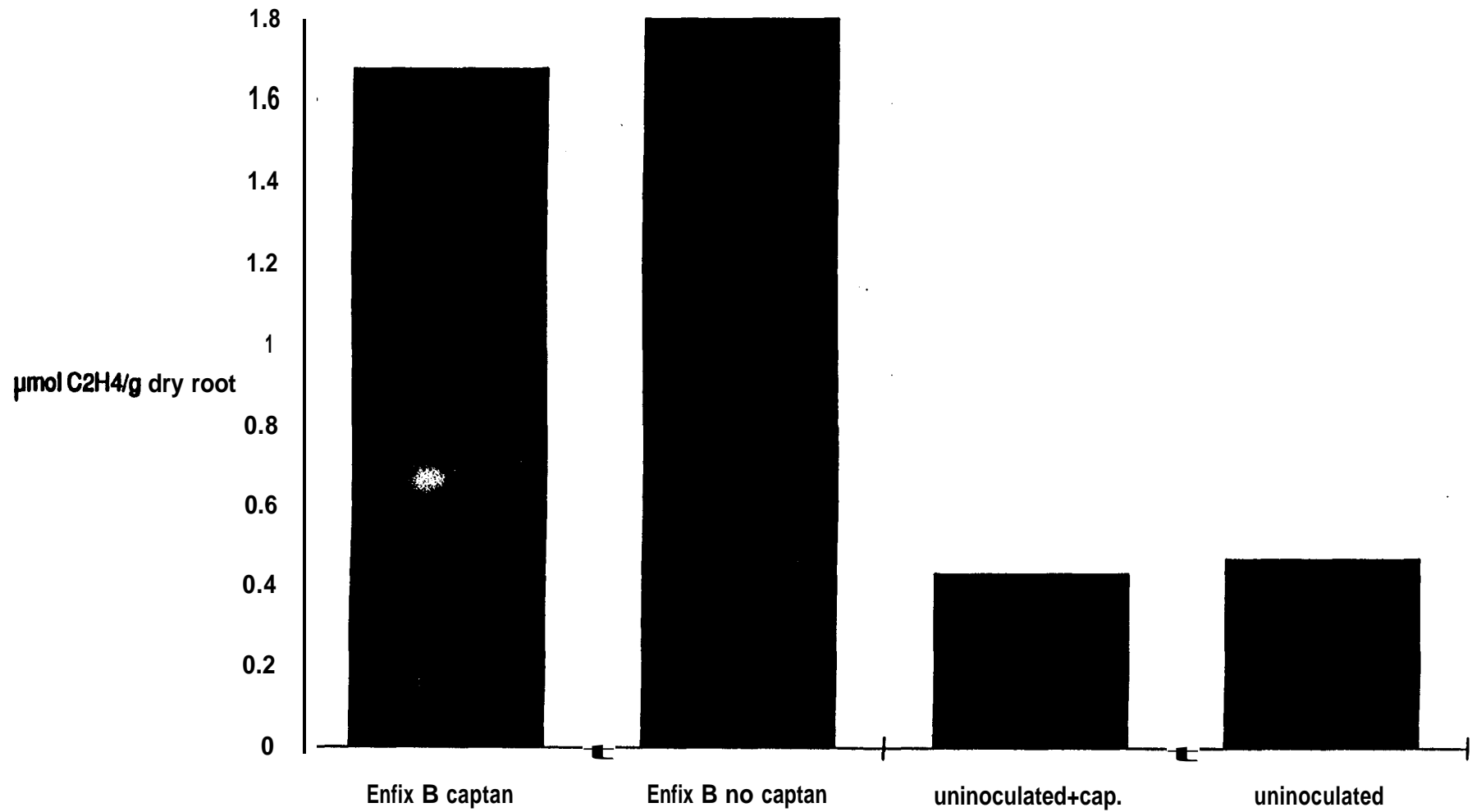


Figure 2.

### Nitrogen Fixation activity of bean root nodules

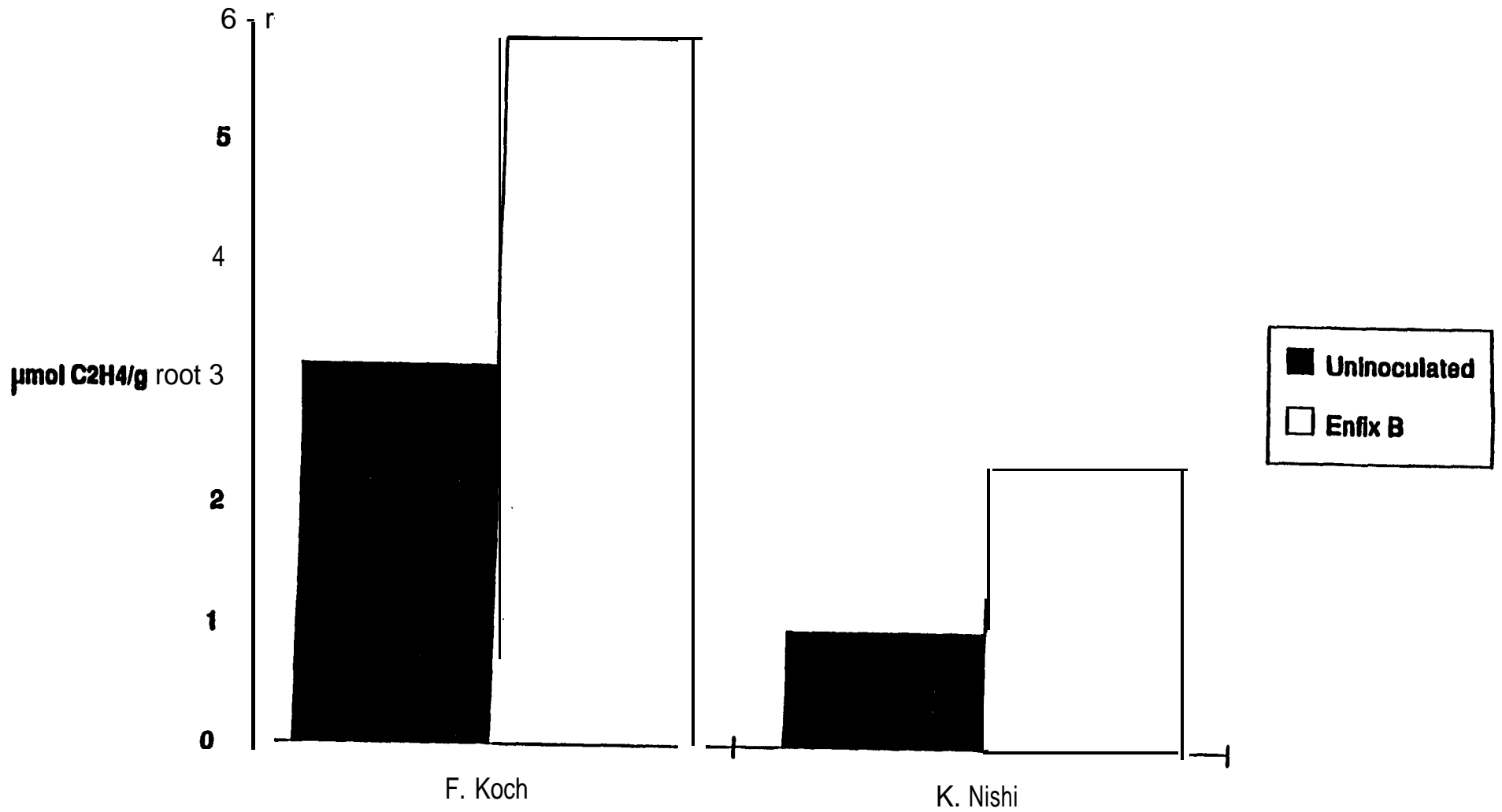


Figure 3.