Liquid rhizobial inoculants for lentil and field pea

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Abstract

The traditional peat legume inoculants are viewed by western Canadian farmers as being difficult to apply. Accordingly, more "user-friendly" inoculants are being developed to ensure more wide-spread inoculation of legumes. A liquid inoculant developed by LiphaTech was evaluated as a carrier for Rhizobium leguminosarum strains 99A1 for lentil and 128C56G for pea. These two strains survived at titres exceeding log 8.0 per mL for ten months at 5°C and there was no loss of viability during shipping and handling. The Prairie Agricultural Machine Institute (PAMI) determined that the liquid inoculant gave a very flowable and uniform coverage of the seeds when applied through a grain auger and various types of seeding equipment. Liquid inoculant for pea and lentil resulted in yields equal or better than those observed for the traditional peat-based inoculant.

Introduction

Symbiotic N_2 fixation occurs following the association of an effective strain of Rhizobium and its host plant. Depending on the legume, up to 60% of the plant's N requirement may be met by the conversion of atmospheric N_2 by the rhizobia to usable N forms for the plant (Innovative Acres Report, 1987). Many western Canada soils do not contain sufficient numbers of Rhizobium to establish effective nodulation, leaving the plant dependent on deriving its N requirement from the soil. Inoculating seed with rhizobia insures that a large and effective population is available in the rhizosphere of the seedling allowing symbiosis to proceed immediately and meet the plants N requirement without interruption in its growth.

The success of establishing a N_2-fixing legume crop is dependant on the carrier system used, the rhizobial strains and the inoculation practices. The carrier must support a large viable population of Rhizobium for several months in a suitable physiological state to establish a symbiotic association with the host seedling. The carrier must be conducive to the rhizobia's ability to be effective in a variety of soil types, environmental conditions and it must be amenable to general agronomic practices (Paau, 1991).

Rhizobium has been applied to seed for several decades in a peat-based carrier. It has proven to be the most effective carrier as a result of the sustaining characteristics peat possess for microorganisms such as its high water holding capacity, high cation exchange (ie. buffering) capacity and abundant nutrient content (Nethery, 1989). In current farming practice seed is treated with one of several sticking agents (Elegba and Rennie,

1984) followed by application of the peat to the seed while it is in the seed box or as it is moved by auger into seeding equipment. Peat-based inoculation practices in western Canada were recently reviewed and it was concluded that although peat was a very good carrier of Rhizobium, the inoculation procedures are generally time consuming, messy and impractical when sowing the large quantities of seed typically sown in this region (P.A.M.I., Food and Farm Report 91-01-03, 1991).

A liquid rhizobial soybean inoculant was developed by Lipha Tech. We viewed this technology as a means of overcoming the difficulties of inoculating Rhizobium onto large quantities of seed. To accomplish this ESSO created new packaging for its lentil and pea inoculants. This study examines the shelf life and efficacy of two liquid inoculants Enfix-L, R. legumniosarum 99A1 for lentils and Enfix-P, R. leguminosarum 128C56G for field pea.

Materials and Methods

Inoculant System

Packaging and a delivery system for the liquid inoculant was developed by ESSO. It was determined that the optimum system for western Canadian farmers would be one that could inoculate 1333 kg (50 bu) of seed. To do this 4 L of inoculant had to be maintained in a physiologically active state for a minimum of 3 months.

Strains

Strains, R. leguminosarum 99A1 and R. leguminosarum 128C56G, used in this study, have demonstrated superior N_2 fixation and yield of lentil and field pea, respectively (Innovative Acres Reports, 1986, 1987 and 1988, Bremer et al., 1990).

Both strains were prepared in peat and liquid-based formulation at LiphaTech, Milwaukee, U.S.A. The strains in the liquid formulation were packaged into plastic bladders of 375 mL (packaging developed at LiphaTech) and 4 L sizes.

The viable counts of R. leguminosarum in the liquid formulation were determined periodically by removing a sample by syringe through a silicon septum on the bags, serially diluting the sample and spreading aliquots of the diluent onto yeast extract-mannitol agar. Colony forming units were counted after 3 days incubation at 25°C. Field pea and lentil were inoculated with strains 128C56G (287 days old) and 99A1(296 days old),respectively, and planted into a mixture of vermiculite and perlite. The pots were placed in a greenhouse and watered twice weekly with Hoaglands N-free nutrient solution. After 4 weeks, the plants were removed from the pots and the roots were inspected for nodule development.

Inoculation trials

Experiments to examine the feasibility of a liquid rhizobial inoculum for lentils and peas were carried out at the Prairie Agricultural Machinery Institute (P.A.M.I.),Humbolt, SK. Farm scale equipment such as a 100 bu bin to hold and release the seed and a auger (40ft by 7 in. operating at one-half speed) were used. The inoculum flowed through a hose (1.5 m long) from the bladder to a tube inserted just above the boot of the auger and coated the lentil and field pea seed as it was carried up the auger to collect in the box of a grain truck. Seed was treated at the recommended rate suggested by the manufacture. Inoculated field pea and lentil seed was collected and planted (24 and 48 hours after inoculation) in pots containing vermiculite and perlite, and placed in the greenhouse. The plants were removed from the pots four weeks later and the tap root nodules were counted.

Field sites

Nine Saskatchewan farmers compared field pea inoculant (strain 128C56G) prepared in the liquid to that of the peat-based formulation. Seed was inoculated according to the manufactures recommendations (peat: 3.2kg/800kg seed; liquid: 4L/1333 kg seed). The cultivars of pea included in this study were Victoria, Trapper, Radley, and Express. All procedures and equipment for inoculation and seeding were according to the farmers normal practices. Each farmer planted the two treatments on two 16 ha plots. Approximately 7 weeks after planting 10 plants from each treatment were removed and nodule development was evaluated. It was not possible to have a similar plot left uninoculated.

Research sites

Research experiments with lentils and field pea were planted in 1991 and 1992 for the purpose of comparing crop vigour, nodule development and grain yield following inoculation with the liquid- and peat-based formulations. Nitrogen fixation was compared between treatments using the acetylene reduction technique (Hardy, Burns and Holsten, 1973). Field sites for lentil (cultivar Laird) and field peas (cultivar Express and Radley) were chosen with two criterion in mind; the site must be in an area where the crop has been traditionally grown and the soil N level must be approximately 20 kg/ha. Field peas and lentils were mechanically planted into plots containing 5 treated rows 2.5 by 6 m using a custom built plot seeder (Fabro Ltd.,Swift Current, SK.) and harvested using a Winterstieger plot combine. Experimental design was a randomized complete block with 8 and 4 replications in 1991 and 1992, respectively. The treatments included uninoculated seed, lentil and pea seed treated with 99A1 or 128C56G, respectively, in the peat and liquid formulations. Statistical analysis was done using MSTAT-C.(Crops and Soil Sciences,Michigan State University, East Lansing, MI) by analysis of variance. Treatment mean separations were done by calculating the LSD at $p \leq 0.05$ level of significance.

Results

A. Formulation/Packaging: Strain Survival

The population of 128C56G and 99A1 exceeded log 8 in the 375 mL (data not shown) and 4 L plastic bladders after nine months (Fig. 1a and b). The shoots of field pea treated with inoculum from the plastic bags, 375 mL and 4 L, were dark green and the crown region of the root and lateral roots were well nodulated.

B. P.A.M.I. Inoculation Trials

Inoculation of lentil and field pea seed using the ESSO packaging and typical farm equipment demonstrated that our inoculation procedure was compatible with commonly used seeding equipment. Treated seed glistened with a moist coating and "coned up" as it collected in the truck box. Within 15 minutes the appearance of the seed returned to that prior to inoculating and showed no seed adhesion. In greenhouse experiments both crops were well nodulated and only a slight reduction in tap root nodule number was observed on plants from seed that was planted 48 hours after inoculation (Table 1). The lateral roots of the lentils and field pea were also nodulated.

Field experiments: A. Farm trials

All of the farmers indicated that the liquid formulation application time was reduced and was much easier to apply to seed than the peat-based formulation . All of the cultivars of field pea inoculated with the liquid and peat-based formulations were nodulated on the crown region of the root indicating that the nodulation commenced soon after germination of the crop (Table 2). Four of the farmers reported measured yields obtained from the two treatments (Fig.2) while the five remaining farmers visually estimated the yield of field peas. The results of these trials indicated that inoculation of field pea with the liquid- or the peat-based formulation gave similar yields (Fig. 2).

Field experiments: B. Research Trials

Lentil vigour, nodule rating and nitrogenase activity of lentil (C_2H_2 reduction) were similar when inoculated with either of the formulations and they greatly exceeded those of the uninoculated treatment (Table 3). Inoculation with either the liquid or the peat-based formulation resulted in similar yield of lentils and regardless of the formulation used, these yields were significantly increased over the yield from uninoculated treatments at 7 of 9 field sites (Table 4).

Similar values for N_2 fixation (C_2H_2 reduction) and nodule rating with field peas inoculated with either formulation was observed (Table 5). The amount of nodulation of field pea observed in the untreated plots by the indigenous population of Rhizobium was greater than that of lentil, suggesting that field pea rhizobia are more promiscuous than lentil rhizobia. The lowest values for nodule rating and N_2 fixation (C_2H_2 reduction) were observed with the uninoculated treatments (Table 5). Similar yields of field pea were obtained with the liquid and peat-based formulation (Table 6).

Discussion

Establishing an effective N_2-fixing symbiosis is highly dependant on a carrier delivering a large and efficacious population of Rhizobium to the host plant. For several decades farmers have relied on peat as a carrier of Rhizobium and while it is an effective delivery system it is messy and time consuming. Legume farming practice in western Canadian often involves inoculating sufficient seed for 500 acres (200 ha) or more. To seed this area in peas, 27 tons of seed will have to be inoculated, a task involving two or more people. Sticker is applied to the seed by one person while another adds peat to the moistened seed as it is moved by auger from the seed bin to a truck box or seed hopper. Therefore, it is not surprising that more "user-friendly" inoculants were requested by farmers (P.A.M.I., 1991).

Several factors contribute to a carriers successful maintenance of a large and effective population of Rhizobium. Peat is a component of soil,therefore, it possesses survival factors for microorganisms such as an diverse nutrient supply and high water holding capacity. The liquid formulation was also capable of maintaining a large and effective population of Rhizobium. The population of R. leguminosarum strains 128C56G and 99A1 decreased by log 0.5 within 30 days of packaging in the plastic bladder and then was stable for the next 4 months. Crown and lateral root nodulation of field pea and lentil by inocula from either container was very good suggesting that this formulation maintains the rhizobia in a physiological state capable of establishing a symbiotic association with its host soon after germination. We cannot speculate on the mechanisms by which the rhizobia are surviving in the plastic bladder at this time, however, it is clear that our understanding of the survival of these organisms under these conditions is lacking.

The liquid formulation was amenable with commonly used farm equipment. The pea farmers reported that application of the liquid inoculant was easy and less time consuming than the peat-based inoculant. In addition, strain 128C56G nodulated four cultivars of pea equally well.

The lentil trials were planted at sites where the farmers have included lentils in their crop rotation. Inoculation with R. leguminosarum 99A1 was required at seven of the nine sites for effective nodule development and N_2 fixation. These results indicate that the population of Rhizobium that nodulate lentils do not survive at levels required for effective nodulation in western Canadian soils.

Peas present a greater challenge. The majority of pea production in western Canada occurs in the black soil zone where the N transformation rates are generally high (R. Karamanos, personal communication) and therefore the benefits of inoculation are not always observed. In addition, there was an effective indigenous population of Rhizobium at some of the field sites. indicating that these soils may maintain an effective Rhizobium population. However, as the results indicate this is not always consistent

Over one million acres of peas and lentils are planted in western Canada. Farmers 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 promote more farmers to include legumes in their planting schedule.

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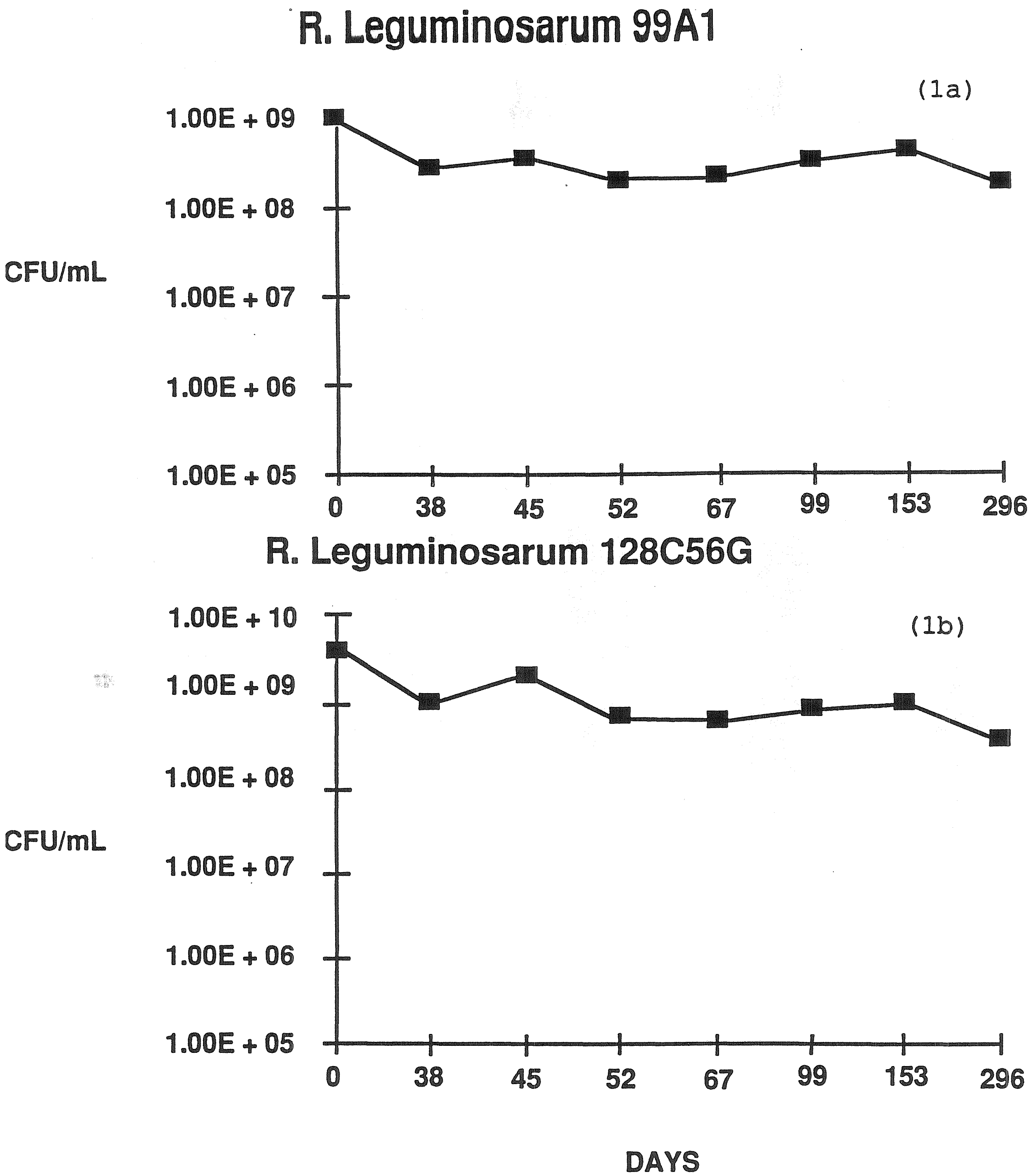

Figure 1.Survival of R. leguminosarum 99A1 (1a) and 128C56G (1b) in a 4L of liquid formulation.

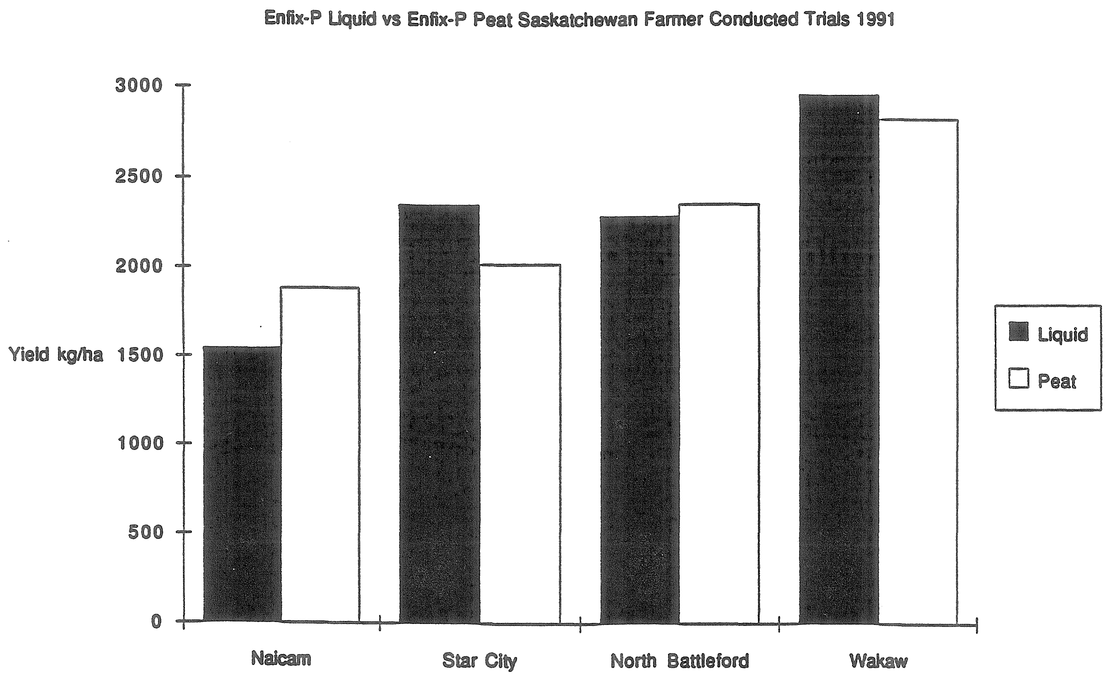

Figure 2. Effect of liquid inoculant on yield of pea at four Saskatchewan farms.

Table 1 Nodule counts on tap roots of field pea and lentil inoculated with liquid rhizobial carrier.

Crop	Number of nodules/tap root[1]		
	0 h	24 h	48 h[2]
Field pea	12.7 ± 1.0 (11)[3]	15.6 ± 0.7 (14)	11.2 ± 0.9 (15)
Lentil	15.2 ± 0.5 (13)	15.8 ± 0.8 (13)	11.1 ± 0.1 (15)

[1] Number of nodules per plant ± S.E.

[2]. Time following treatment to planting

[3] Number in brackets refers to the number of plants harvested.

Table 2 Nodulation of various cultivars of field pea by R. leguminosarum 128C56G.
Effect of formulation on crown nodulation and nodule rating.

Location	Cultivar	Previous Crop	Treatment	%Crown Nodulation[1]	Nodule Rating[2]
Radisson	Victoria	Fallow	Peat	100	5
			Liquid	100	5
Payton	Trapper	Wheat	Peat	80	3
			Liquid	60	2
Nipawin	Trapper	Wheat	Peat	90	4
			Liquid	80	4
Star City	Radley	Flax	Peat	100	4.5
			Liquid	80	3
Tisdale	Trapper	Canola	Peat	100	5
			Liquid	100	5
N.Battleford	Victoria	Wheat	Peat	100	3
			Liquid	100	3
Wakaw	Express	Canola	Peat	100	5
			Liquid	100	5
Naicam	Trapper	Flax	Peat	100	2.5
			Liquid	100	2.5
Melfort	Radley	Barley	Peat	100	3.5
			Liquid	100	3.5

Ten plants per treatment examined.
1. Per cent crown root nodulation calculated from the total number of plants examined per treatment.
2. Nodule rating: 0-no nodules to 5-excellent nodulation.

Table 3 Effect of inoculation with Rhizobium leguminosarum 99A1 in peat or liquid formulation on vigour and nodule rating and % crown nodulation of lentil (cv. Laird) in the field.

Treatment	Vigour Rating[1]	Nodule Rating[1]	µmol C2H4/g dry root[2]
Peat	3.5	3.1	6.10±1.17
Liquid	3.3	3.0	7.22±0.65
Uninoculated	1.9	1.2	0.41±0.37

1.Vigour and nodule rating use a scale of 0 (no growth or nodulation) to 5 (excellent growth and nodulation). Vigour rating represents mean of 3 sites (Kindersely, Raymore and Rosetown 2). Nodule rating represents the mean of 5 sites (Kindersely, Soverign, Raymore, Success and Moose Jaw).
2 Acetylene reduction data from 1992 field site only.

Table 4. Inoculation of R. leguminosarum 99A1 on lentil. Effect of formulation on grain yield in 1991 and 1992 field experiments.

	Yield (kg/ha)								
	Raymore	Rosetown	Moosejaw	Success	Sovereign	Success	Outlook	Raymore	Rosetown
Treatments	1991					1992			
99A1 Liquid	1270	2174	2691	2717	3118	1014	1241	1931	2195
99A1 Peat	1219	1916	2381	2573	3275	1318	1123	2151	2369
Uninoculated	876	1370	2230	2038	3122	656	1275	950	1022
LSD	339	257	433	356	382	413	457	747	506

Table 5 Effect of inoculation with R. leguminosarum 128C56G in peat and liquid formulation on nodule rating and nitrogen fixation (C_2H_2 reduction) on pea (cv Radley and Express)

Treatment	Nodule rating[1]		µmol C_2H_4/g dry weigth root[2]
	1991	1992	
Peat	3.5	3.8	8.46±3.54
Liquid	2.6	3.6	8.43±1.11
Uninoculated	1.3	2.5	5.89±1.39

1: Mean nodule rating for 1991 obtained from experiments at Redwated, North Battleford, and Brandon. Mean nodule rating for 1992 obtained from experiments at Redwater, Pigeon Lake and Saskatoon.
2: Acetylene reduction from the 1992 experiments

Table 6. Inoculation of R. leguminosarum 128C56G on field pea.
Effect of formulation on grain yield in 1991 and 1992 field experiments.

	Yield (kg/ha)			
	Redwater	Pigeon Lake	Saskatoon	Redwater
Treatments	1991	1992		
128C56G Liquid	4376	2194	3361	4143
128C56G Peat	4339	3058	3009	3856
Uninoculated	4200	2680	2804	3370
LSD	524	938	776	637