Inoculant Formulation and Fertilizer Nitrogen Effects on Field Pea: Crop yield and Seed Quality

G. W. Clayton, W. A. Rice, N. Z. Lupwayi, A. M. Johnston, G. P. Lafond, C. A. Grant and F. Walley

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Introduction

According to Statistics Canada, the cropping area for field pea (Pisum sativa) in Western Canada has increased from 25 000 ha in 1976 to 1 335 000 ha in 2001. As part of the production system, field peas require inoculation with *Rhizobium leguminosarum* bv. *Viceae*, particularly, when grown for the first time in rotation. Appropriate rhizobial inoculation and fertility management can increase field pea seed yield and improve yield stability in western Canada.

The most common form of legume inoculation is application of cultures of *Rhizobium* spp. to seed in liquid formulation or, usually, by first impregnating the *Rhizobium* in peat (peat formulation) and applying it to the seed[22]. Field pea growers have popularised liquid inoculant, mainly because of the ease of use[13]. Soil inoculation methods with granular inoculant, applied in the seed row, have been successful for large-seeded legumes such as faba bean (*Vicia faba* L.), soybean (*Glycine max)*, peanut (*Arachis hypogaea*) and dry bean (*Phaseolus vulgaris* L.)[8,9,10]. Alternative inoculant delivery systems may be preferable to traditional methods of inoculation[2,3,4]. Successful inoculation of field pea is important for optimising plant N nutrition, decreasing yield variability, and increasing economic return[9].

While the survivability of inoculants is quite good, failures generally result from environmental stresses such as dry seedbed conditions or acid soils[21]. Farmers have responded to inoculation failures by applying higher rates of inorganic nitrogen to insure against economic losses. Fertilizer N is known to adversely affect nodulation and N_2 fixation of legumes[5,23]. However, small 'starter' doses of fertilizer N are sometimes beneficial to plant development and subsequent nodulation and N_2 fixation[16], especially when initial nodulation is reduced or delayed. Improved inoculant delivery systems insure against inoculation failure by increasing the number of rhizobia at seeding and reducing the mortality rate once applied to the soil[23].

As part of this study, the effects of soil inoculant (granular) and seed-applied inoculant (peat powder or liquid), used with and without urea-N application on field pea, were investigated in the Peace River region of Western Canada. Soil characteristics and further experimental details of the study are presented in Clayton et al. (2003).

The relative ranking of different inoculant formulations and their effects on field pea nodulation and N_2 fixation were: granular inoculant > peat inoculant > liquid inoculant in the Peace River region of Alberta[6]. Nitrogen fertilizer reduced both nodulation and nodule weight. This paper reports on how these methods of inoculation and N fertilizer translated into pea seed yield and quality.

RESULTS AND DISCUSSION

Overall Effects on Biomass

At low applied N rates, field pea biomass was significantly higher for soil-applied inoculants, as compared to seed-applied inoculants. Soil-applied inoculants resulted in 15, 18, 9 and 0% higher pea biomass yield at the flatpod stage than seed-applied inoculant at 0, 20, 40 and 80 kg N ha-1, respectively. When averaged over all N rates, soil-applied inoculant resulted in 17, 50, and 56% higher pea seed yield than peat inoculant, liquid inoculant, or the uninoculated check, respectively. Soil-applied inoculant increased the proportion of the biological yield converted to seed compared to seed-applied inoculant.

Seed protein concentration was increased by 12 and 15% when inoculant was soil-applied compared with seed-applied or uninoculated pea, respectively. Without N fertilizer, soil-applied inoculant increased field pea biomass, seed yield, and protein concentration and contributed to increasing yield stability compared with seed-applied inoculant.

Effects on Biomass Production at Flatpod Stage

Significant environment, inoculant formulation, N rate, inoculant formulation x N rate, and inoculant formulation x environment effects were detected for pea biomass dry matter yield determined at the flatpod stage of growth (Table 1). But when averaged over all N rates, biomass at this stage was not significantly different between inoculated treatments and the uninoculated check. However, soil-applied inoculants resulted in 10% greater field pea biomass than seed-applied inoculants. There was no difference in the biomass between the seed-applied inoculants. The differences that occurred between soil and seed-applied inoculants were due to high biomass accumulation with granular inoculant at Beaverlodge South in 1996 and Beaverlodge North in 1995.

Table 1. Significance of ANOVA of biomass, seed yield, harvest index and seed protein concentration at harvest as affected by inoculant formulation, N rate and environment

Source of Variation	df	Biomass ^{z}	Seed yield	Harvest index	Seed protein concentration
Environments (E)	5	**	ns	**	**
Formulation (F)	3	*	**	*	**
F x E	15	**	**	*	ns
Nitrogen rate (N)	3	**	ns	ns	ns
N x E	15	ns	ns	*	ns
F x N	9	**	ns	*	ns
F x N x E	45	ns	*	ns	ns

^{z} Biomass dry matter at flatpod stage of field pea

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively; ns is not significant

Peat inoculants had a field pea biomass dry matter yield that was equal to the pea crops grown with liquid inoculant at all sites; except Beaverlodge North in 1995. At this site, peat inoculant significantly increased pea biomass yield by 15% compared to the liquid inoculant. The results from inoculation methods were averaged over the N rates, which likely caused significant contrasts at only two of six site/years. Studies have found that application of N fertilizer to uninoculated field pea maintained shoot biomass at flowering similar to the granular inoculant control[19].

The inoculant formulation x N rate interaction was explained by higher field pea biomass with soil-applied inoculants at 0 and 20 kg urea-N ha^{-1}, but not at 40 or 80 kg N ha^{-1}, than the seed-applied inoculants or the uninoculated check. Soil-applied inoculants resulted in 15, 18, 9 and 0% higher pea biomass yield than seed-applied inoculants at 0, 20, 40 and 80 kg urea-N ha^{-1}, respectively. Seed inoculation resulted in 5, 7, -4 and 1% higher biomass yield than uninoculated pea at 0, 20, 40 and 80 kg N ha^{-1}, respectively. Pea biomass increased with N application rates of 80 kg N ha^{-1} with peat inoculant and 40 or 80 kg N ha^{-1} with liquid inoculants or the uninoculated field pea compared to 0 kg N ha^{-1}.

With granular inoculant, N fertilizer did not significantly increase field pea biomass compared to granular inoculant without N fertilizer. Reports indicate that granular inoculants increased growth of fababean at an early stage relative to seed-applied inoculant[8]. Results from this study suggest that the N requirements of field pea for biomass production were met by N_2 fixation when granular inoculant was soil applied, similar to previous findings by other scientists[9]. Additional fertilizer N was required to increase biomass in field pea that had seed-applied inoculants, or no inoculant at all.

Effects on Seed Yield

A significant inoculant formulation, environment x inoculant formulation, inoculant formulation x N rate x environment effect was detected for seed yield (Table 1). The seed yield of field pea was in the order: granular inoculant > peat inoculant > liquid inoculant = uninoculated. Seed yield was 17 to 67% higher from inoculated pea compared with uninoculated pea at five of six site/years. When averaged over all the sites, seed yield was 31% higher when field pea was inoculated than uninoculated. Soil-applied inoculant, compared to seed-applied inoculant, significantly increased the seed yield by 10 to 45%.

The seed yield from pea with peat inoculant was significantly increased by 4 to 66% compared with liquid inoculant. When averaged over all sites and N rates, granular inoculant resulted in 17, 50 and 56% higher pea seed yield than peat inoculant, liquid inoculant and the uninoculated check, respectively. When seed-applied inoculant was compared, peat inoculant increased yield by 28% compared to liquid inoculant. Other researchers found no difference in pea seed yield when peat inoculant and liquid inoculant were compared in Saskatchewan trials[13].

In other Alberta studies[18], field pea yields were significantly increased by rhizobial granular inoculation in 41% of the trials with an average yield increase of 14%, compared to uninoculated check. Yield response was 29% if soil NO_3-N was less than 20 kg N ha^{-1}, and 10% if soil NO_3-N was more than 20 kg N ha^{-1}. All of the sites had NO_3-N levels above 20 kg N ha^{-1} and an average yield increase of 56% from granular inoculant compared to the uninoculated check were observed. Applying granular inoculant in the seed row of common beans increased yield by 50% compared to an uninoculated bean control[20]. In this study, the trials were direct-seeded and yields were higher than those reported in other Alberta studies, which may have affected the percent yield response[18]. The inoculation formulation x N rate x environment interaction indicated that there were site differences. Inoculant formulation x N rate interactions occurred only at Fort Vermilion in 1995 and at Beaverlodge South in 1995.

At Fort Vermilion in 1995, soil-applied inoculants resulted in a significant yield increase at 0 N kg ha^{-1} compared with seed-applied inoculants, which was equal to the uninoculated check. Fertilizer applied at 80 kg N ha^{-1} increased seed yield of pea with peat inoculant, liquid inoculant, and the uninoculated check compared to pea without N fertilizer at Fort Vermilion in 1995.

However, application of N fertilizer decreased seed yield of field pea with granular inoculant by as much as a third compared to pea with granular inoculants and no N fertilizer.

Consequently, there were no differences in seed yield of pea among inoculant formulations at 40 or 80 kg urea-N ha^{-1}. In field experiments conducted in Alberta and Saskatchewan, the survival of *R. leguminosarum* bv. *viciae* on pea seed soon after inoculation, and colonization of the rhizosphere of pea plants, were clearly shown to be lower with liquid inoculant than with peat powder or granular inoculant in one of two years[13]. It is possible that the unfavorable environmental conditions resulted in poor *Rhizobium* survival from seed-applied inoculant and that precipitation later in the growing season enhanced the effect of the N fertilization in seed-applied inoculants and uninoculated treatments.

Less effective nodulation and N_2 fixation occurred with seed-applied inoculant than soil-inoculated pea[7]. Soil moisture conditions were dry at this site during the period up to the flatpod stage, despite the plots being direct-seeded. Peat powder has been reported to offer some protection from desiccation to the rhizobia under environmental stress[10]. In contrast, liquid inoculant provides relatively little protection to the rhizobia, thus, they are more susceptible to unfavourable environmental conditions, such as excessive heat and desiccation both during and after seeding. It appears that in Fort Vermilion in 1995, granular inoculant provided some protection for rhizobia from the dry conditions but was unable to maintain that protection when dry soil was combined with applied N fertilizer in the seed row.

At Beaverlodge South in 1995, there was no yield response to N fertilizer with granular inoculant. Granular inoculant increased seed yield of field pea compared to liquid inoculant and the uninoculated check at all N rates. Seed yield was significantly higher with peat inoculant than with liquid inoculant when fertilizer was applied at 20 and 40 kg N ha^{-1}, mainly because seed yield was low with the liquid inoculant at these N fertilizer rates.

Inhibition of N_2 fixation, in response to added fertilizer N, has been reported in many studies and it is generally agreed that the symbiotic legume/*Rhizobium* association is most effective in N-limiting systems[1]. Other researchers[26] have proposed that the negative effects of combined N on the legume/*Rhizobium* association typically fall within three classes: 1) the effect on infection events; 2) the effect on nitrogenase activity per unit mass of nodule; and 3) the effect on nodule mass per plant. Although N fertilizer may have had an impact within all three classes, the observation at Fort Vermilion in 1995, that yield declined in the soil inoculation plots in response to increasing N fertilizer rates, suggests that infection events in particular were restricted. Both nodule number and nodule weight were reduced with fertilizer N under the dry soil conditions at Fort Vermilion in 1995[7].

Studies have found that application of N fertilizer depressed pea yield in 3% of the trials conducted and increased seed yield in 24% of the trials but did not detect an interaction between granular inoculant x N fertilizer[20]. Similarly, an inoculant formulation x N rate interaction was not detected at four of six site/years in this study. However, field pea yield could be enhanced by application of 100 kg N ha^{-1} to levels equal to that of a granular inoculant control[17]. In this study, there was no need for application of N at low or high rates when soil inoculation with granular inoculant was used with peas. In some situations, added N at seeding may contribute to increased yield, particularly when field pea had seed-applied inoculants or they were uninoculated. However, added N fertilizer contributed more to vegetative growth than to seed formation.

Effects on Harvest Index

There were significant environment, inoculant formulation, inoculant formulation x environment, N rate x environment and inoculant formulation x N rate effects for pea harvest index (Table 1). Harvest index of inoculated pea was significantly higher than that of uninoculated pea. Soil-applied inoculant resulted in a significantly higher harvest index than seed-applied inoculants.

Table 2. Effect of inoculant formulation and N rate on pea harvest index averaged over six environments

	N rate (kg ha^{-1})				
	0	20	40	80	
Inoculant formulation	Harvest index				Mean
Granular	0.38	0.35	0.39	0.34	0.37
Peat powder	0.34	0.37	0.36	0.32	0.35
Liquid	0.35	0.33	0.31	0.33	0.33
Uninoculated	0.35	0.31	0.31	0.31	0.32
Mean	0.36	0.34	0.34	0.32	

$LSD_{0.05}$ for Inoculant formulation = 0.02
$LSD_{0.05}$ for Nitrogen rate = 0.02
$LSD_{0.05}$ for Inoculant formulation x Nitrogen rate interaction = 0.04

Harvest index was variable across site/years as indicated by the environment x inoculant formulation interaction. Soil-applied inoculant increased the harvest index significantly compared to seed-applied inoculant and the uninoculated check at Fort Vermilion in 1995. Soil and seed-applied inoculant resulted in a significantly higher harvest index than the uninoculated check at Beaverlodge South in 1995 and 1996. There were no differences in harvest index among the treatments at Beaverlodge North in 1996. There were fewer significant contrasts for harvest index than there were for seed yield.

For example, soil-applied inoculant resulted in a higher harvest index in two of six site/years compared to seed-applied inoculant. The same comparison resulted in a significantly higher seed yield with soil-applied inoculant than seed-applied inoculant in all six site/years. Consequently, soil-applied inoculant increased both seed yield and straw yield, as the proportion of the biological yield converted to seed was similar to seed-applied inoculant at four of six sites/years. At the other two sites, soil-applied inoculant increased both seed yield and straw yield compared to seed-applied inoculant, as the proportion of the aboveground biomass converted to seed was increased compared to seed-applied inoculant. Factors other than N alone appeared to be influencing harvest index at these two sites.

The inoculant formulation x N rate interaction occurred because harvest index was significantly reduced at 80 kg N ha^{-1} compared to harvest index of pea without N fertilizer with granular inoculant (Table 2). Nitrogen fertilizer significantly decreased harvest index at all applied rates compared to harvest index without N fertilizer in the uninoculated check. The proportion of the biological yield converted to seed declined with the application of N fertilizer in the uninoculated check, despite the increase in biomass with N fertilizer at the flatpod stage. Harvest index was similar with liquid inoculant at all applied N fertilizer rates except at 40 kg ha^{-1}. The nitrogen x environment interaction indicated that harvest index was variable in site/years. N fertilizer reduced harvest index at four of six site/years and increased harvest index at one of six site/years. Reports that nitrogen application increased biomass production of field pea but did not necessarily increase seed yield have been made[15]. In this study, N fertilizer increased biomass with little benefit to seed development, particularly with the liquid inoculant and the uninoculated check.

Effects on Seed Protein Concentration

Significant effects of environment and inoculant formulation were detected for seed protein concentration (Table 1). Inoculated field pea had 7% higher seed protein concentration than uninoculated pea. Soil-applied inoculant resulted in a 12% increase in seed protein concentration compared to the seed-applied inoculant. Scientists, have indicated that there was generally a beneficial effect of inoculation on protein concentration and that granular inoculant resulted in a greater increase than liquid inoculant[15]. There was no significant difference in protein concentration between peat inoculant and liquid inoculant. Applying N up to 80 kg ha^{-1} did not affect protein concentration, as the N fertilizer likely ended up in the plant biomass rather than to the seed biomass. The effect of N rate on protein concentration was generally small where both increases and decreases could occur[18].

Soil-applied inoculant resulted in greater N accumulation between flatpod and maturity, total N accumulation, and N_2 fixation than seed-applied inoculant and the uninoculated check[7]. The higher N_2 fixation and N accumulation during podfilling from soil-applied inoculant likely contributed to the higher protein concentration compared to seed-applied inoculant or the uninoculated check.

It is possible that lateral and crown nodules can achieve greater N_2 fixation than crown nodules alone[11,24], which may contribute to the increased seed yield and protein concentration.

Conclusion

In summary, soil-applied inoculant increased pea seed yield by 17-56% and protein concentration by 12-15% compared to seed-applied inoculants, and the uninoculated check. Of the seed-applied inoculants, the peat inoculant typically was superior to the liquid inoculant in enhancing seed yield. In some cases, N fertilizer enhanced field pea biomass and seed yield in inoculant treatments that were typically less effective (i.e., uninoculated control and liquid inoculant). Whereas, N fertilizer application were either ineffective or negative to seed yield in inoculant treatments that favoured relatively high yields (i.e., granular inoculant, and in most instances, peat inoculant).

Granular inoculant can improve field pea yield stability, particularly under environmental stress where reduced rhizobia mortality rate is critical to mitigate the stress. Future studies should further define inoculant delivery systems that impact nitrogen nutrition and yield stability. Studies may include rate and placement of soil-applied granular and liquid inoculants, fertilizer-granular inoculant mixtures and granular inoculant carrier types.

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Self-Study Exam

1. As part of the production system, field peas require inoculation with *Rhizobium leguminosarum* bv. *Viceae*, particularly.________________.
   - **a. When grown for the first time in rotation**
   - b. When grown after a crop removes high levels of nitrogen from the soil
   - c. When grown in a field with low moisture levels
   - d. When following a legume crop

2. The survival of inoculants in the soil can be negatively affected by ____________
   - a. Poor moisture conditions
   - b. Low pH
   - c. High organic matter
   - **d. Two of the above**
   - e. All of the above

3. At low applied N rates, field pea biomass was significantly higher for seed-applied inoculants, as compared to. soil-applied inoculants.
   - a. True
   - **b. False**

4. Results from this study suggest that the N requirements of field pea for biomass production were met by N_2 fixation when granular inoculant was soil applied, similar to previous findings by other scientists
   - **a. True**
   - b. False

5. Looking at the research data, soil-applied inoculant resulted in greater N accumulation between flatpod and maturity, ______________, and ____________ than seed-applied inoculant and the uninoculated check.
   - a. Total N accumulation and harvest index
   - **b. Total N accumulation and N_2 fixation**
   - c. N_2 fixation and harvest index
   - d. N_2 fixation and inoculant survivability

6. Soil-applied inoculant increased pea seed yield by 17-56% and protein concentration by 12-15% compared to seed-applied inoculants, and the uninoculated check
   - **a. True**
   - b. False

7. __________ inoculant, compared to __________ inoculant, significantly increased the seed yield by 10 to 45%.
    a. Soil applied innoculant, seed applied inoculant
    b. seed applied inoculant, peat applied
    c. Soil applied innoculant, peat applied
    d. Peat applied, soil applied
8. With granular inoculant, studies showed that the addition of N fertilizer significantly increased field pea biomass as compared to granular inoculant without N fertilizer
    a. True
    b. False

9. According to this study, the relative ranking of different inoculant formulations and their effects on field pea nodulation and N_2 fixation were: _____________
    a. Peat inoculant > granular inoculant > liquid inoculant
    b. Liquid inoculant > peat inoculant > granular inoculant
    c. Peat inoculant > liquid inoculant > granular inoculant
    d. granular inoculant > peat inoculant > liquid inoculant

10. Looking at the overall effect of inoculants on biomass, soil-applied inoculant decreased the proportion of the biological yield converted to seed compared to seed-applied inoculant.
    a. True
    b. False