The effect of a nodulating enhancer on marrowfat pea plant development, seed yield and size

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Abstract

Marrowfat pea (*Pisum sativum*) seed is grown in New Zealand predominantly for human consumption as a snack food throughout Asia. Previous overseas studies on the addition of growth promoting legume inoculants containing *Rhizobium leguminosarum* bv. *viciae* have provided variable results when used in various types of peas. Such inoculants are available in commercial formulations and are used in many pulse growing regions. A single trial was established in 2011-12 in Canterbury, New Zealand to evaluate the effect of the commercial legume inoculant Nodulator® Granular Legume Inoculant which was applied in-furrow at 6 kg/ha to three marrowfat pea varieties. The addition of this inoculant at sowing did not significantly affect (P=0.05) plant establishment, length of stem to first node, final plant height, number of nodes to first pod, number of pods per plant or lodging. In addition there was no significant effect on grain yield or seed size. The only significant results obtained were between pea varieties when measurements were analysed across inoculant treatments. These significant differences can be attributed to genetic difference between the marrowfat pea varieties.

Additional keywords: *Pisum sativum*, Nodulator® Granular Legume Inoculant, *Rhizobium*

Introduction

Legume plants are capable of forming symbiotic relationships with *Rhizobium* bacteria which enables nitrogen fixation to occur. Rhizobacteria occur naturally in many soils (indigenous), but, maybe absent or in insufficient numbers depending on crop history (Greenwood, 1965). Palmer and Young (2000) found that genetic diversity of rhizobacteria was higher under arable fields in contrast to relatively undisturbed grasslands. Legumes respond to *Rhizobium* inoculation by developing unique structures on their roots and occasionally stems, called nodules (Hirsh, 1992; Masson-Boivin *et al.*, 2009). There has been a large amount of research done on plant growth promoting rhizobacteria (PGPR) including the isolation of specific bacterial strains for various legume species (Dileep Kumar *et al.*, 2001; Hynes *et al.*, 2008; Zahir *et al.*, 2008; Kidaj *et al.*, 2012). Extensive work has been done on white clover (*Trifolium repens* L.) seed inoculation in New Zealand which has been summarised by Lowther and Kerr (2011) but there is no reported work on peas in New Zealand.

The addition of inoculants containing *Rhizobium leguminosarum* biovar *viciae* has been shown to be beneficial in some studies involving field peas (Clayton *et al.*, 2004a; 2004b) although others have found variable or little benefit to be gained (Chanway *et al.*, 1989; McKenzie *et al.*, 2001; Kutcher *et al.*, 2002, Chemining'wa and Vessey, 2006; Tsigie *et al.*, 2011). Brockwell *et al.* (1980) and Clayton *et al.* (2004a; 2004b) found inoculant formulation type was important, with granular soil applied formulations incorporated with the seed at sowing, tending to be better than peat powder or liquid formulations in peas. This could be due to a better distribution of inoculant within the seed furrow and/or the higher quantity of inoculant that can be applied compared with seed coating. Granular inoculants have been used in the USA and Canada for many years. They have the advantage of ease of storage, handling and application (Herridge, 2009).

Marrowfat peas (*Pisum sativum* L.) are a field pea generally grown for human consumption. Dried peas are produced for markets including Japan and Southeast Asia where they are consumed as a snack food and in the United Kingdom (UK) where they are sold as 'mushy peas'. Most of the marrowfat pea seed is produced in Canada, UK and New Zealand (D.R. Storrier pers. comm., 2011). Premiums are paid for high quality colour and uniform large-sized grain (PIDG, 2008). Agronomically marrowfat peas are generally mid-season maturing peas with first flowers occurring on nodes 15-16. Traditional varieties have been 'leafed' types although more recent releases have been 'semi-leafless'. The marrowfat pea produces a large, irregular grain with a green cotyledon and a clear seed coat (PIDG, 2008).

The addition of any type of growth promoting legume inoculant is not common practice when growing marrowfat pea seed in New Zealand. The objective of this trial was to evaluate the effect of a commercially available pea inoculant, Nodulator® Granular Legume Inoculant (Group E & F) manufactured by Becker Underwood Inc., Canada, on seed yield and other agronomic characteristics in three commercial marrowfat pea varieties grown in Canterbury, New Zealand.

Materials and Methods

In 2011-12 a single field trial site was established on a light Lismore stony silt loam soil (Kear *et al.*, 1967) in Winchmore, Canterbury, New Zealand (43° 48' 32" S, 171° 42' 19" E). The trial site had been cropped for two years (brassica followed by carrots) after coming out of long-term pasture. Three cultivars (cv. Midichi, Midlea and 22-5) were sown either with Nodulator® Granular Legume Inoculant or without, giving a total of six treatments. Treatments were replicated three times in a randomised complete block design.

Untreated seed was sown on 12 October 2011 without fertiliser using a belt-cone plot seeder. Seeding rate was calculated separately for each cultivar according to thousand seed weight (TSW), with an estimated germination and emergence of 90% and a target population of 100 plants/m^2. Inoculated treatments had Nodulator® Granular Legume Inoculant (NGLI) added to the seed on the sowing cone immediately prior to planting. Each inoculated treatment received the recommended label rate of 6 kg/ha of NGLI (Lot 312, expiry date February 2012). Each gram of NGLI is reported to contain 1×10^8 viable cells of *Rhizobium leguminosarum* bv. *viciae*. Plots were 8 m in length and consisted of nine rows with 15 cm spacing (10.8 m^2). A buffer of 0.5 m between plots was allowed to reduce any risk of inoculum transfer and to ensure any lodged treatments did not interfere with others. Plot yields were calculated from an area of 1.5 m ×8 m (12 m^2) to allow for the additional buffer space between plots (outside row effect) to ensure yield was not over estimated.

Crop management was the same for all plots. Weeds were controlled with Gardoprim applied at 2 l/ha (a.i. 500g/l terbuthylazine) post-plant pre-emergence. Buffer zones were sprayed with Roundup 360 at 10 ml/l (a.i. 360 g/l glyphosate) using a knapsack and further hand weeding was done as required to ensure a clear area surrounded each plot. Dovetail at 1 l/ha (a.i. 5g/l lambda-cyhalothrin + a.i. 100g/l pirimicarb) was applied to plots for aphid control on 17 November 2011. Chess at 200 g/ha (a.i. 500g/l pymetrozine) was applied on 13 December 2012 also for aphid control. No fertiliser or fungicide treatments were applied. The trial was irrigated as required with a gun RM Super Rain 100. It was a relatively wet growing season so only two applications were required of approximately 25 mm each.

Plant populations were estimated when plants were at the 3-4 leaf stage, with four counts of 1 linear m conducted in each plot. Plant height was measured in the centre of each plot at the end of flowering (5-6 January 2012) and a visual assessment of lodging was also made. Bird damage to some plots was seen on 3 February 2012, at which time a visual assessment of pod damage was recorded and all plots were then covered with bird netting. Just prior to desiccation for harvest, 10 plants per plot were removed and the following measurements were made: length of stem to first pod, number of nodes to first pod and number of pods per plant. No measurement of nodule numbers, size or viability was made.

All plots were desiccated on 23 February 2012 with Reglone applied at 3 l/ha (a.i. 200 g/l diquat) in 400 l/ha water. Plots were all harvested on 28 February 2012 with a Wintersteiger Elite plot harvester. The entire plot sample was collected and dried in a commercial dryer. Entire samples were then dressed using a Mini Pektus 80 seed cleaner, with a 5.1 × 19 mm slotted screen over a 7.5 mm round-holed screen. The machine dressed (MD) sample was weighed and the moisture content (MC) determined using a Draminski moisture meter. Final MD yields were corrected to14% MC. Seed size was determined by counting the number of seeds in a 10 g sub-sample per plot and scaled up to seeds/100 g. The larger the seed size count the smaller the peas are in size, this is the standard customer method for assessing seed size quality.

Statistical analysis was completed using computer software SAS 2008 (version 9, second edition, SAS Inc., North Carolina, USA). Analysis of variance was conducted on individual agronomic measurements. All data was normally distributed except for lodging and bird damage scores. Normal data was analysed using a general linear model with significant differences distinguished by the least significant difference (LSD) method (P=0.05). Lodging and bird damage scores were analysed using non-parametric ANOVA.

Results

Plant establishment for all three cultivars was acceptable and near the trial target of 100 plants/m^2. The addition of Nodulator® Granular Legume Inoculant (NGLI) did not significantly affect plant establishment. When establishment data was analysed across NGLI treatments there was a significant variety affect. Variety 22-5 (109 plants/m^2) had significantly higher plant establishment than Midichi (92 plants/m^2) while Midlea (102 plants/m^2) was intermediate.

Marrowfat pea growth as measured by final plant height (m), stem length to first pod (m), number of nodes to first pod and number of pods per plant were not significantly affected by the addition of NGLI across varieties. When data was analysed across NGLI treatments there were significant differences between varieties for all agronomic measurements (Table 1). Lodging scores taken prior to flowering were not normally distributed. A non-parametrical analysis of variance showed that variety had a significant effect on the amount of lodging but NGLI had no significant effect. When lodging data is analysed across NGLI treatments, variety 22-5 (14%) demonstrated significantly more lodging than either Midichi (8%) or Midlea (7%) respectively.

Table 1: Pre-harvest plant agronomic measurements analysed across Nodulator® Granular Legume Inoculant treatments for each marrowfat pea variety.

Variety	Final plant height (m)	Stem length to first node (m)	No. nodes to first pod	No. pods per plant
Midichi	1.04a	0.61a	15a	11b
Midlea	0.96a	0.62a	14a	8a
22-5	1.19b	0.70b	18b	12b
$LSD_{(0.05)}$	0.11	0.07	1	2

Means within a column with the same letter are not significantly different ($LSD_{(0.05)}$).

With respect to seed yield NGLI did not have a significant treatment effect although variety did (Table 2). The trial site did incur some bird damage prior to harvest. Bird damage was not significantly affected by NGLI treatments. Bird damage analysed across NGLI treatments showed a significant difference between varieties (Table 2). Bird damage appeared to be related to variety maturity and not NGLI. Bird damage would have resulted in some yield loss in both Midichi and Midlea, but the amount could not be quantified in this trial. It is possible that the significant differences in yield between varieties may not be real, but a result of bird damage.

Finally seed size was not affected by the incorporation of NGLI. When data is analysed across NGLI treatments there was once again a significant variety effect. Variety Midichi produced significantly smaller seed (280 peas/100 g) compared with either 22-5 (265 peas/100 g) and Midlea (253 peas/100 g), the latter two not being significantly different from each other (Table 2).

Table 2: Lodging, grain yield, bird damage and seed size analysed across Nodulator® Granular Legume Inoculant treatments for each marrowfat pea variety.

Variety	Lodging (%)	Grain yield (kg/ha at 14% MC)	Bird damage (% pods damaged)	Seed size (no. peas/100g)
Midichi	8a	3813ab	12b	280b
Midlea	7a	3517a	13b	253a
22-5	14b	4431b	4a	265a
$LSD_{(0.05)}$	5	631	4	12

Means within a column with the same letter are not significantly different ($LSD_{(0.05)}$).

Discussion

At this one trial site which had no known history of pulse production, there was no evidence that the inclusion of NGLI significantly altered (either positively or negatively) any of the agronomic traits measured in any of the three marrowfat pea cultivars trialled. These findings are similar to those of Kephart and Opena (2003) who found that not only Nodulator® but also five other commercially available nitrogen inoculants did not significantly influence pea establishment, test weight, MC, thousand seed weight, yield or protein content in Montana, USA. There was some suggestion the Kephart and Opena (2003) results may have been affected by hot, dry growing conditions (it was a dryland pea trial). However, this trial was conducted under excellent soil moisture conditions and irrigation was available when required, so drought was not a contributing factor to these results. Grain yield was very good across all treatments and comparative to local commercial yields.

Variety Midichi was bred in New Zealand by The New Zealand Institute for Crop and Food Research and released in 1999 (PVR, 2012). Midichi is an early-mid maturing, medium height, leafed marrowfat pea with good, even seed size and shape with a proven yield record in Canterbury. Variety Midlea is of similar breeding to Midichi and is also a leafed type. It has been grown in Canterbury on a lesser scale and has not undergone PVR registration. Midlea is very similar in appearance and growth habit to Midichi, but, consistently produces larger sized pea seed. The yield potential of Midlea is similar to Midichi. Variety 22-5 is the newest marrowfat pea variety to be released. It does have Midichi in its parentage but is a semi-leafless, mid-late maturing, tall marrowfat pea with good pea seed size and high yield potential, which has not undergone PVR registrations (unpublished data, Midlands Seed Ltd; A. Russell pers. comm., 2010). Not surprisingly, there were differences between varieties due to their genetic differences.

There are a number of factors that can reduce the efficacy of *Rhizobium* bacteria including: using the wrong species/strain of *Rhizobium*, delays between inoculation and seeding, improper inoculant storage and environmental conditions during growing (Anonymous, 2007). The product used in this trial is commercially available and formulated for peas and lentils (Becker Underwood Inc., 2011). It was stored prior to use in a chiller (4°C) in its original packaging and added to the seed immediately prior to sowing, four and a half months before its expiry date.

Kutcher *et al.* (2002) had mixed success with using a commercial granular inoculant in peas. In their trial set (up to 14 sites) they found inoculation improved pea establishments 14% of the time (and reduced it 7% of the time). While pea grain yield was significantly improved in 46% of their trials and grain protein was increased significantly in 38% of trials. They concluded that the variability in effects of inoculants was strongly influenced by local soil and environmental conditions. It is possible that prevailing environmental conditions resulted in poor survival of inoculated *Rhizobium leguminosarum* bv. *viciae*, although the growing season was considered to be above average across a wide range of crops in 2011-12 and overall yields from this trial would be considered commercially acceptable. The trial area had been fallowed over winter and conditions at sowing were very good, with adequate soil moisture.

The addition of nitrogen based fertilisers is reported to reduce nodule numbers and N_2 fixation (Clayton *et al.*, 2004; Achakzai, 2007); however no fertiliser was applied to the trial. Neither the amount of nodulation nor nodule quality within treatments was assessed. Further, soil samples were not analysed for soil nitrogen levels or levels of indigenous *Rhizobium leguminosarum* bv. *viciae*, so *Rhizobium s*urvival cannot be confirmed in this trial. These will need to be included in any future inoculation trials.

Conclusions

The Department of Primary Industries in Australia takes a conservative approach and recommends that all legumes are inoculated at sowing. Their reason for this is that there are far fewer problems with unnecessary inoculation than with not using inoculants when they are needed (Herridge, 2009). A recent review of white clover inoculation in New Zealand (Lowther and Kerr, 2011) recommends addition of an inoculant should only be considered in three situations; undeveloped grasslands, virgin pastoral land cleared from scrub or when following 10 years of consecutive maize production. Even then the likelihood of a measureable response is considered unlikely.

Although the evidence is overwhelming in this trial that the addition of Nodulator® Granular Legume Inoculant did not improve any aspect of marrowfat pea production on current commercial varieties, the data is limited to one site and the trial did not confirm whether nodulation occurred. Whether further work is required is debatable, but if more trials were to be completed then the following improvements to trial methodology would be advised: trials should include a wider range of commercial inoculants evaluated at more sites with more detailed nodulation assessments including nodule numbers, quality and quantification of *Rhizobium* levels in the soil. Soil fertility analysis during the trial period with an emphasis on nitrogen levels would also be helpful.

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