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Project number:	FV 428
Project leader:	Dr Lea Wiesel, PGRO
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

The application of starter fertilisers containing phosphorus to peas at drilling showed potential to increase yields. Any effects of fertiliser applications on rhizobial populations in soils remain to be investigated.

Background

Pea yields have reached a plateau in many areas over recent years and one option to boost yields is the application of starter fertilisers. Starter fertilisers contain phosphorus which is important for root development and nitrogen fixation by rhizobia. Nitrogen fixation not only delivers nitrogen to the pea crop but also increases soil nitrogen for subsequent crops. Some starter fertilisers, however, contain nitrogen which can be damaging to rhizobia populations with negative impacts on the pea crop and soil nitrogen contents. Thus, it is important to maintain soil conditions that sustain healthy rhizobia populations in soil. The project therefore investigates whether application of starter fertilisers increases pea yield and whether applications of starter fertilisers have an effect on rhizobia populations in soil.

Summary

Starter fertilisers with and without nitrogen have been applied to three different pea crops. In early drilled crops, yield was not affected but in mid and late drilled crops the applications of both Primary P (with nitrogen) and Microstar (without nitrogen) lead to higher yields. Strongest increases of nearly 4 t/ha have been achieved in mid drilled crops by the application of Microstar at 10 kg/ha. Results are observations only and due to the lack of replication statistical analysis could not be performed. So far, it cannot be concluded that the application of starter fertilisers impacts on rhizobia populations. This is due to the lack of a reliable method to assess population sizes. A pot test to assess rhizobia populations has now successfully been developed and tested on a small subset of samples. In mid drilled crops, rhizobial population sizes in plots that had received Primary P or Microstar at 12.5 kg/ha did not differ from untreated plots. Sample numbers are too small to draw final conclusions and all soil samples taken in both field seasons will be tested.

Financial Benefits

Yield improvements due to fertiliser application varied strongly with field site and have been obtained in un-replicated trials but results indicate a positive effect on yield by starter fertilisers at mid and late drilled sites. Application of starter fertilisers costs approximately £25/ha. On average, the price per tonne of peas is £345. An increase of pea yield of just 73 kg/ha will result in breaking even and any yield increase of greater than 73 kg/ha will result in an economic benefit for pea growers. Results on environmental impacts are inconclusive so far and rely on assessment of the remaining soil samples.

Action Points

Action points have yet to be identified.

SCIENCE SECTION

Introduction

Vining peas rely on the symbiotic relationship with rhizobia that provide nitrogen to the plant which is vital for plant growth and yield. A result of this relationship is a crop without the need for nitrogen applications and an increase in soil nitrogen for the subsequent crop. In many areas pea yields have reached a plateau and one possible solution to increase yield is the application of starter fertilisers. Phosphorus promotes root growth and is essential for root nodulation to occur. Applying starter fertiliser containing phosphorus to peas holds the potential to increase yield and help to maintain healthy populations of rhizobia in soils. The more rhizobia there are in the soil, the greater the potential for root nodulation and the greater the amount of fixed nitrogen available to the pea plant. A potential issue with application of starter fertilisers to peas is that some of the fertilisers contain nitrogen. Nitrogen is potentially damaging to rhizobial populations thereby not only reducing nitrogen fixation in the pea crop but also leaving reduced levels of soil nitrogen for the subsequent crop.

The project investigates the influence of phosphorus starter fertilisers on pea yields and the effects of these fertilisers (with and without nitrogen) on rhizobial populations in soils.

Materials and methods

Three field trials were established in 2015 using early, mid and late commercial vining pea varieties, Novella, Geneva and Serge, respectively (Table 1). Three rates of two different starter fertilisers were applied to each crop at drilling (Table 2) and an unfertilised control was maintained. Starter fertiliser Primary P which contains nitrogen was applied at 7.5 kg/ha, 10 kg/ha and 12.5 kg/ha as was starter fertiliser Microstar that does not contain nitrogen (Table 3). Each field plot was approximately two hectares in size and was treated, other than the application of starter fertiliser, as a commercial crop.

Table 1: Field trials 2015. Location of field trials, drilling dates and varieties used.

Name	**Location**	**Field**	**Drilling date**	**Variety**
Early	Blossom Hall Farms	BH061	31/3/15	Novella
Mid	Ward Farming	JW038	10/4/15	Geneva
Late	G.H. Emerson	GE132	4/5/15	Serge

Table 2: Rates of starter fertilisers Primary P and Microstar applied at drilling.

Fertiliser	Application	Rates
Primary P	7.5 kg/ha; 10 kg/ha; 12.5 kg/ha	75%; 100%; 125% recommended rate
Microstar	7.5 kg/ha; 10 kg/ha; 12.5 kg/ha	75%; 100%; 125% recommended rate

Table 3: Composition of starter fertilisers Primary P and Microstar.

Fertiliser	Composition
Primary P	40% phosphorus, 11% sulphur oxide, 10% nitrogen, 2% manganese, 2% zinc
Microstar	45% phosphorus pentoxide, 3% magnesium oxide, 0.5% copper, 0.5% manganese

Soil and plant sampling

Soil samples were taken to assess numbers of rhizobia in soils and to perform nutrient analysis. Nutrient analysis was carried out because a fertiliser containing nitrogen was used in a Nitrate Vulnerable Zone and samples were tested by Hill Court Farm Ltd. Soil samples for assessing rhizobia numbers were taken before drilling and at first flower whereas soil samples for nutrient analyses were taken before drilling and after harvest. Soils were sampled across a W shape in each plot for rhizobial analysis and as soil cores to a depth of 90 cm for nutrient analyses.

Twenty plants per plot were sampled at first flower to measure shoot and root dry weights (DW) as well as to visually score nodulation on plant roots. Yields were measured per plot at harvest.

Assessment of rhizobial populations in soils

1. Most Probable Number (MPN)

   The Most Probable Number (MPN) method is described in the Handbook for Rhizobia by Somasegaran and Hoben (1994) and used to calculate viable rhizobia per gram of substrate. The method uses ten-fold dilution series of soil suspensions to inoculate pea plants grown under sterile conditions. Nodules per plants are counted and viable numbers of rhizobia calculated using tables published in the Handbook for Rhizobia

(Somasegaran and Hoben, 1994). Here, after several modifications to the original protocol, peas were grown in boiling tubes containing 20 ml of nitrogen free nutrient agar (Broughton and Dilworth, 1971), inoculated with soil dilutions, grown at 15°C with 16 h light for 5 weeks and assessed for nodulation.

2. Pot tests to assess rhizobia numbers in soils

Peas were grown in pots (5 plants per pot) filled with a sterile 1:1 vermiculite/perlite mixture fertilised with a nitrogen free nutrient solution (Broughton and Dilworth, 1971). One week after plant establishment, soil slurries containing 1 g of soil per 10 ml sterile distilled water (SDW) were added. All treatments were carried out in triplicate. Four weeks after inoculation, nodules per pot were counted and shoot DW measured. Nodules per plant and shoot DW per plant were calculated.

Results

Yield data from the three field trials are shown in Figure 1. Fertiliser application did not affect yields in early drilled plots but application of both fertilisers increased yield in mid drilled plots. In late drilled plots, slight yield increases occurred but mainly due to application of Microstar which does not contain nitrogen. These data are observations only because statistical analysis was hindered due to the lack of replication.

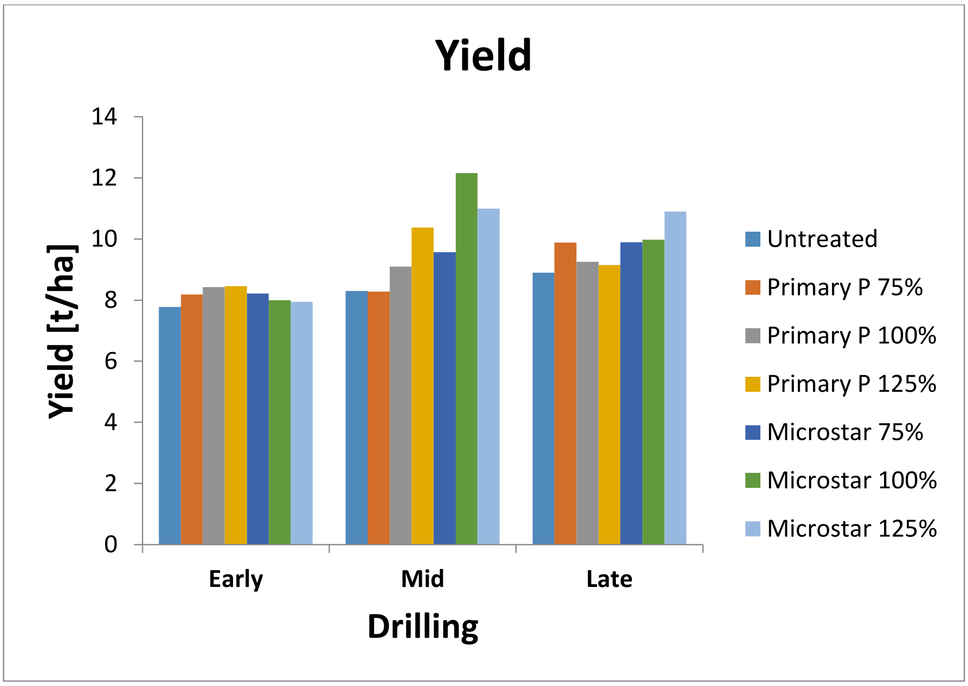

Figure 1. Pea yields from untreated plots and plots that had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show un-replicated yields per plot [t/ha].

Root and shoot DW from 20 plants collected from the field were measured. Root DW was not affected by fertiliser application in early drilled plots (Figure 2). Root DW significantly increased due to Microstar (without nitrogen) application of 10 and 12.5 kg/ha in mid drilled plots (ANOVA, $F_{6,27} = 3.5$, $p < 0.1$). In late drilled plots, application of Primary P (with nitrogen) at 7.5 kg/ha and of Microstar at 10 kg/ha significantly increased root DW in comparison to the unfertilised control (ANOVA, $F_{6,27} = 3.1$, $p < 0.1$).

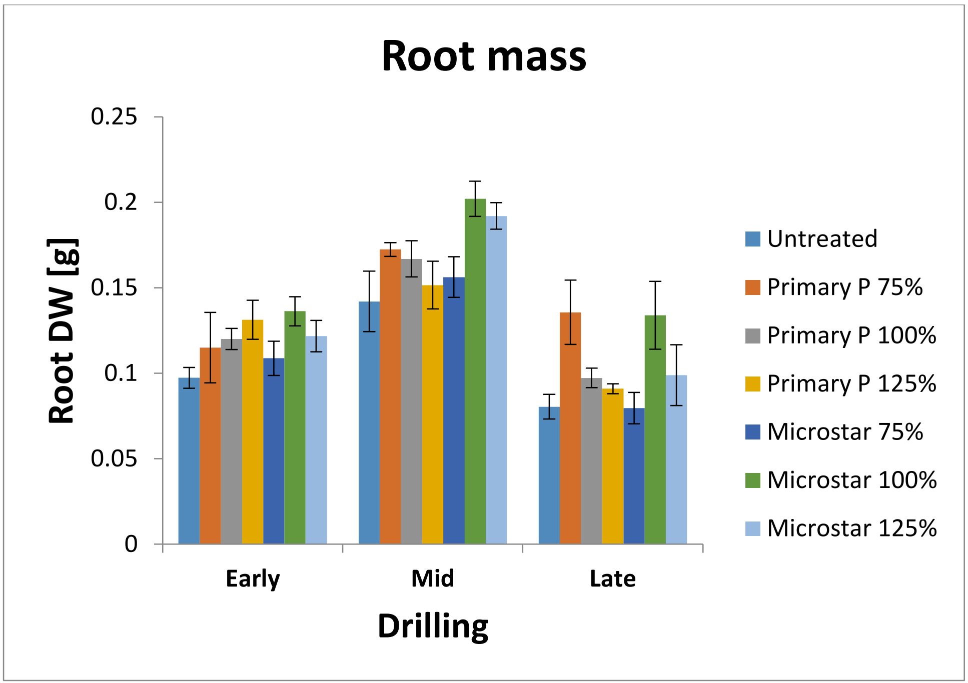

Figure 2. Root DW of plants collected from the different field trials. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values (n = 4) and standard error.

Shoot DW was only significantly affected in late drilled plots where application of Primary P at all three rates increased shoot DW in comparison to the unfertilised control (Figure 3; ANOVA, $F_{6,27} = 3.9$, $p < 0.1$). In early and mid drilled plots, shoot DW was unaffected.

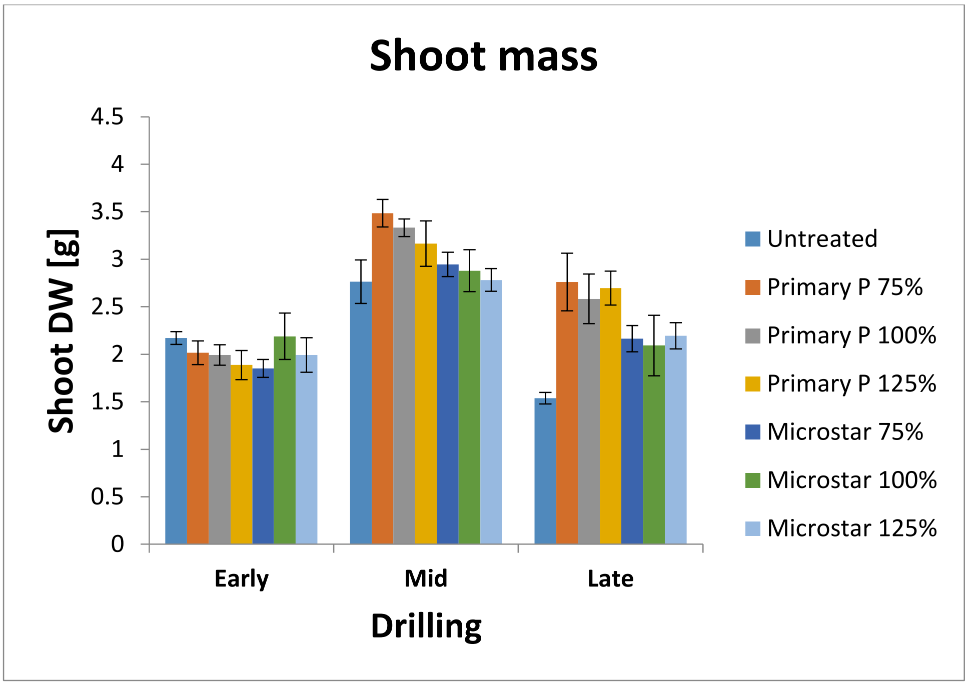

Figure 3. Shoot DW of plants collected from the different field trials. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values (n = 4) and standard error.

Roots of plants collected from the field have been scored for nodule formation on a scale from 0 (no nodules) to 5 (very dense nodulation). All plants were nodulated and in mid and late drilled plots, nodulation did not differ between fertilised and unfertilised plots (Figure 4). In early drilled crops, nodulation rating was significantly higher in plots fertilised with Primary P at 7.5 and 12.5 kg/ha and with Microstar at 10 kg/ha in comparison to unfertilised crops (ANOVA, $F_{6,27} = 4.6$, $p < 0.01$).

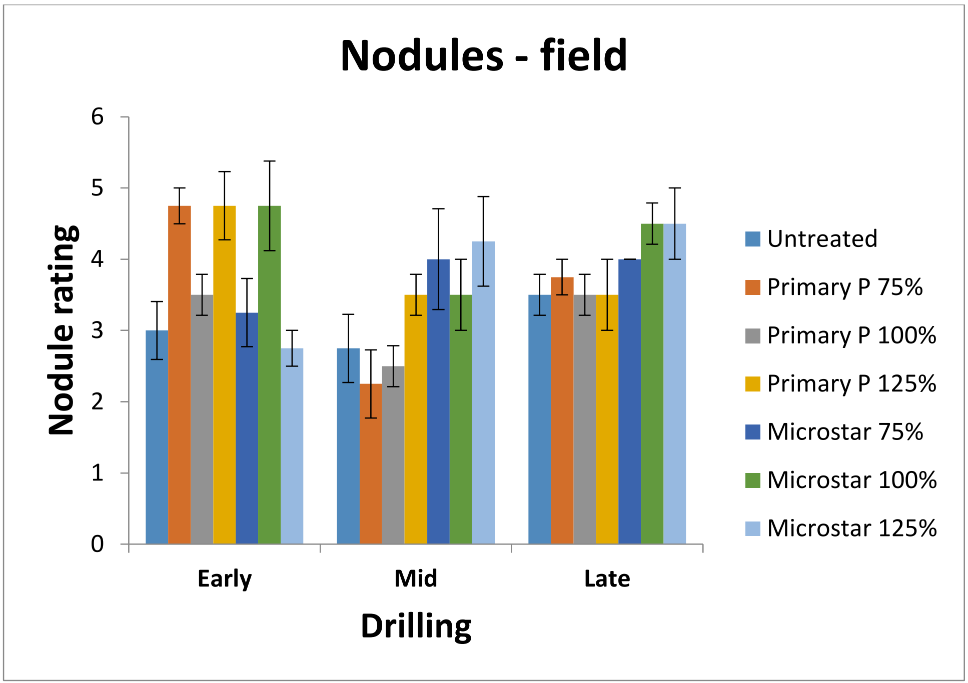

Figure 4. Nodulation of plants collected from the different field trials. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values (n = 4) and standard error.

To test the impact of fertiliser application on numbers of viable rhizobia in soil, the MPN method was initially used. This, however, was unsuccessful. After several modifications to the original protocol after which inoculation method, growing time and growing conditions were improved, nodulation did occur on pea roots but results were inconsistent and unreliable (Table 4). A total of 54 different soil samples collected in 2015 have been tested. Out of these, results from 23 samples were not usable. The MPN method is very strict and requires an unbroken chain of nodulation over the 10-fold dilution series and a full set of nodulation over all four replicates for the lowest two dilution steps. If these conditions are not met, the results are not usable. The results from the successful soil samples varied greatly from 17 rhizobial cells to 1700 rhizobial cells per gram soil. This variation, however, was random and not attributable to treatment effect. The failure of the MPN method was most likely due to two reasons. Firstly, the use of agar as growing medium stopped free movement of rhizobia through the medium and also restricted oxygen flow which is vital for nodulation to occur. Secondly, the size of the tubes used to grow the peas was too small and restricted plant growth. Plant health was not optimal which reduced likelihood of successful nodulation. It was therefore decided to replace the MPN method.

Table 4. Numbers of viable rhizobia per gram soil. 10^X = 10 to the power of X. Not usable = nodulation did not occur in unbroken dilutions or in full sets at the two lowest dilutions and results were therefore unusable for calculation of rhizobia per gram soil. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar).

		MPN [rhizobia per g soil]		
Treatment	**Rep**	**Early**	**Mid**	**Late**
Untreated	1	3.1*10^2	3.1*10^1	1.7*10^1
	2	not usable	3.1*10^1	5.8*10^2
	3		1*10^3	5.8*10^1
	4			3.1*10^1
Primary P 75%	1	3.1*10^1		not usable
	2	5.8*10^1		not usable
	3	not usable		5.8*10^1
	4			3.1*10^1
Primary P 100%	1	not usable	not usable	not usable
	2	5.8*10^1	3.1*10^1	
	3	1.7*10^1		
	4		not usable	not usable
Primary P 125%	1	not usable	1.7*10^1	
	2		not usable	
	3	not usable	not usable	3.1*10^1
	4		not usable	1.7*10^2
Microstar 75%	1	5.8*10^1		1*10^2
	2	not usable	1.7*10^1	5.8*10^1
	3			
	4	not usable		not usable
Microstar 100%	1	not usable	not usable	5.8*10^1
	2	not usable	3.1*10^2	3.1*10^1
	3	1.7*10^1		1*10^2
	4		1.7*10^2	
Microstar 125%	1	1*10^2		1.7*10^3
	2	not usable	3.1*10^1	
	3	not usable	not usable	
	4			

The MPN method was replaced with a pot test in which numbers of nodules on pea roots are counted. This method allows free movement of rhizobia and oxygen flow in the growth medium and enables optimal plant growth. In 2015, a small sub-sample of soils was tested. Soil samples of untreated plots and plots that had received 12.5 kg/ha of either fertiliser in the mid drilled crop were tested. All four field replicates were tested in technical triplicate. Nodulation was successful and hundreds of nodules developed per pot. Nodulation, however, did not statistically vary due to fertiliser application (Figure 5). Shoot DW as a measure for effective nitrogen fixation was also measured but was not affected by soil treatment either (Figure 6).

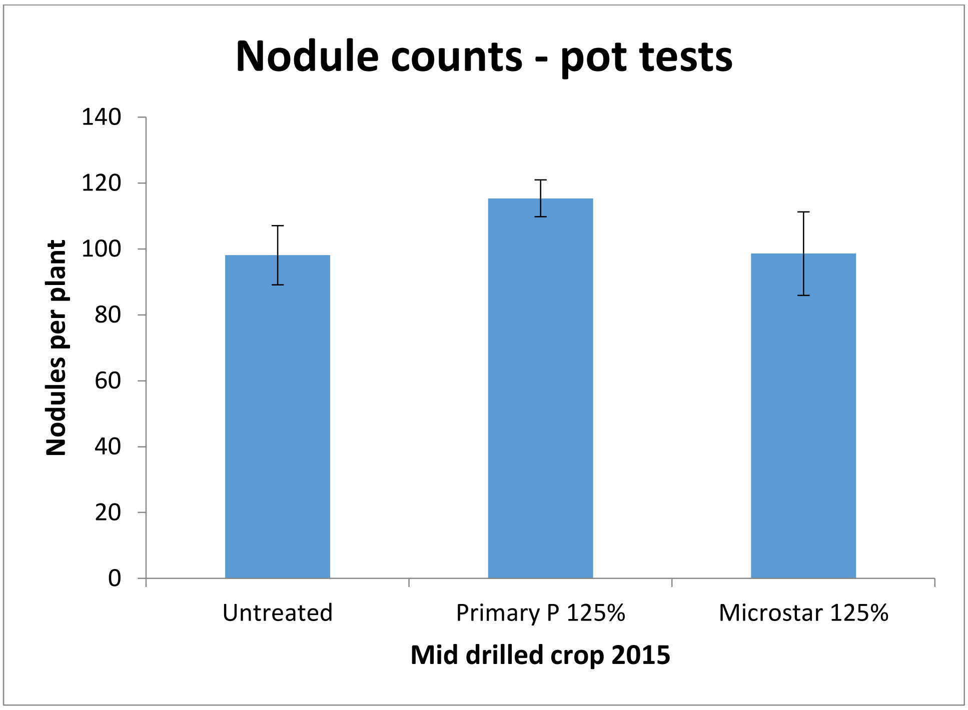

Figure 5. Number of nodules per plant. Plants were inoculated with soil from untreated field plots, and field plots that had either received Primary P or Microstar at 12.5 kg/ha. Soil samples had been collected from mid drilled fields in 2015. Data show mean values (n = 12) and standard error.

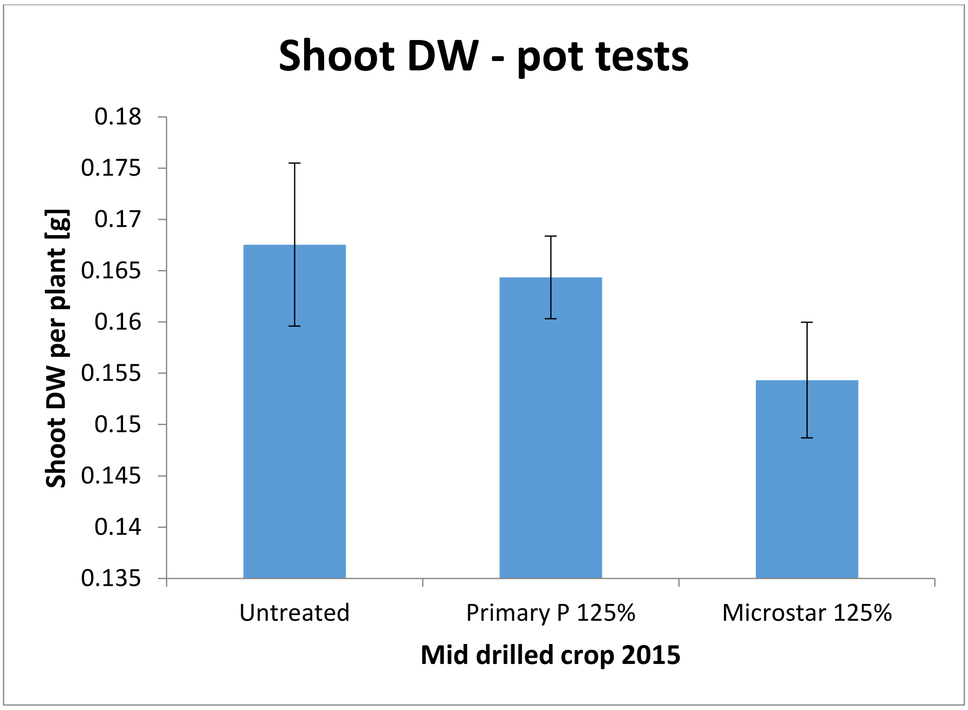

Figure 6. Shoot dry weight per plant. Plants were inoculated with soil from untreated field plots, and field plots that had either received Primary P or Microstar at 12.5 kg/ha. Soil samples had been collected from mid drilled fields in 2015. Data show mean values (n = 12) and standard error.

Nutrient data (potassium, phosphorus and magnesium) and pH were measured before drilling and after harvest. In the early and late drilled fields, only untreated plots and plots that had received Primary P were analysed (Appendix 1). Soil type, nitrate, ammonium, available nitrogen, potentially mineralisable nitrogen (PMN), organic matter (OM) and total nitrogen (tN) were measured at all three field sites before drilling (Appendix 2).

Discussion

One objective of the project is to investigate the effects of applying phosphorus starter fertiliser with or without the addition of nitrogen to peas with the aim to achieve yield increases. In 2015, application of starter fertilisers did increase pea yield in mid and late drilled crops with greatest increases seen in mid drilled crops. Microstar, the starter fertiliser without nitrogen, seemed to have strongest impacts with a yield increase of 4 t/ha in the mid drilled crop that had received 10 kg/ha. These results suggest that application of starter fertilisers to peas holds the potential to enhance yield. However, results are observations only because they have obtained from un-replicated field trials and statistical analysis could not be performed.

Another objective of the project is to investigate the effects phosphorus starter fertiliser with or without the addition of nitrogen on rhizobia populations in soils. Phosphorus is essential for nodulation and could potentially boost rhizobia populations in soil. Nitrogen, however, can be detrimental for rhizobia populations. Over the course of the project, it has not been possible yet to investigate these potential effects due to the lack of a reliable method to assess rhizobia populations. This has now been rectified. A pot test to assess nodulation potential of rhizobia populations in different soil samples has been developed. This test will be used to test all soil samples taken over both years of the project to answer whether any of the starter fertilisers has an effect on rhizobia populations. First results indicate that populations are not affected but sample size is too small to draw reliable conclusions. Furthermore, soil samples that had been taken from fields with different phosphorus indices will also be tested to give more insight in the relationship of soil phosphorus and rhizobia populations.

Conclusions

- Application of starter fertilisers to peas holds the potential to increase yields.
- A method to assess rhizobial population sizes has been developed.
- Effects of starter fertilisers on rhizobia populations remain to be investigated.

Knowledge and Technology Transfer

Agronomy training CCC Agronomy (February 2015)

Agronomy training Saffron Waldon (February 2015)

Cereals 2015 (June 2015)

Dengie Crops grower meeting (February 2015)

Fen Peas Ltd Grower meetings (March and October 2015)

Hutchinsons Agronomy meetings (12 meetings during 2015)

Legume panel meetings (February and November 2015)

PGRO and Syngenta Roadshows (6 meetings in January and February 2015)

PGRO Crop Protection course (February 2015)

PGRO Pulse Open Day Stubton (July 2015)

PGRO Vining pea Open Day Nocton (June 2015)

TerresInovia and PGRO meeting (June 2015)

References

Broughton, W.J. and Dilworth, M.J. (1971) Control of leghaemoglobin synthesis in snake beans. Biochemical Journal 125: 1075-1080.

Somasegaran, P. and Hoben, H.J. (1994) Handbook for rhizobia. Methods in legume-*Rhizobium* technology. Springer Verlag New York, Inc.

Appendices

Appendix 1. pH, phosphorus (P), potassium (K) and magnesium (Mg) measurements. Soil samples were taken before drilling and after harvest. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar).

Early drilled	Pre drilling							Post harvest						
Treatment	pH	Phosphorus (P)		Potassium (K)		Magnesium (Mg)		pH	Phosphorus (P)		Potassium (K)		Magnesium (Mg)	
		mg/l	Index	mg/l	Index	mg/l	Index		mg/l	Index	mg/l	Index	mg/l	Index
Untreated	7.84	27	3	208	2+	92	2	7.89	56	4	433	4	112	3
Primary P 75%								7.78	31	3	266	3	69	2
Primary P 100%								7.86	39	3	354	3	79	2
Primary P 125%								7.83	36	3	317	3	94	2
Microstar 75%														
Microstar 100%														
Microstar 125%														

Mid drilled	Pre drilling							Post harvest						
Treatment	pH	Phosphorus (P)		Potassium (K)		Magnesium (Mg)		pH	Phosphorus (P)		Potassium (K)		Magnesium (Mg)	
		mg/l	Index	mg/l	Index	mg/l	Index		mg/l	Index	mg/l	Index	mg/l	Index
Untreated	8.05	18	2	297	3	119	3	8.26	17	2	331	3	134	3
Primary P 75%								8.2	16	2	404	4	159	3
Primary P 100%								8.28	16	2	299	3	112	3
Primary P 125%								8.28	16	2	314	3	123	3
Microstar 75%								8.38	16	2	273	3	121	3
Microstar 100%								8.07	12	1	408	4	120	3
Microstar 125%								8.22	12	1	539	4	141	3

Late drilled	Pre drilling							Post harvest						
Treatment	pH	Phosphorus (P)		Potassium (K)		Magnesium (Mg)		pH	Phosphorus (P)		Potassium (K)		Magnesium (Mg)	
		mg/l	Index	mg/l	Index	mg/l	Index		mg/l	Index	mg/l	Index	mg/l	Index
Untreated	7.66	9	0	305	3	218	4	8.25	8	0	193	2+	115	3
Primary P 75%								8.03	12	1	235	2+	196	4
Primary P 100%								7.91	18	2	259	3	155	3
Primary P 125%								8.21	11	1	237	2+	181	4
Microstar 75%														
Microstar 100%														
Microstar 125%														

Appendix 2. Soil type, nitrate, ammonium, available nitrogen, potentially mineralisable nitrogen (PMN), organic matter (OM) and total nitrogen (tN) measurements. Soil samples were taken before drilling and after harvest at all three field sites. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P).

Drilling	Sampling time	Treatment	Depth (cm)	Soil type	Nitrate (kg/ha)	Ammonium (kg/ha)	Availiable N (kg/ha)	PMN (kg/ha)	OM (%)	tN (%)
Early	Pre drilling	Whole field	0-30	ZL	25.2	0.8	25.9	110.8	2.42	0.133
			30-60	ZL	36.9	3	39.9			
			60-90	ZL	37.9	2	39.9			
Early	Post harvest	Untreated	0-30	ZCL	33	2.6	35.6	102.1	3.03	0.166
			30-60	ZCL	41.6	2.6	44.2			
			60-90	ZCL	40.5	1.2	41.8			
Early	Post harvest	Primary P 75%	0-30	ZCL	33.4	1.4	34.8	72.3	2.62	0.145
			30-60	ZL	37.9	0	37.9			
			60-90	ZL	43.6	0	43.6			
Early	Post harvest	Primary P 100%	0-30	ZCL	29.5	0.9	30.4	62.1	2.76	0.167
			30-60	ZL	37	1.7	38.7			
			60-90	ZL	30.9	0	30.9			
Early	Post harvest	Primary P 125%	0-30	ZCL	20.5	1.5	22	50.2	2.62	0.122
			30-60	ZCL	28.5	0	28.5			
			60-90	ZCL	30.8	0.1	30.9			
Mid	Pre drilling	Whole field	0-30	ZL	24	1.3	25.3	114.2	2.61	0.098
			30-60	ZL	43.7	0	43.7			
			60-90	ZL	43.9	0.4	44.3			
Mid	Post harvest	Untreated	0-30	ZCL	15.6	3.1	18.6	79.7	3.23	0.136
			30-60	ZL	28.9	1.7	30.6			
			60-90	ZL	41.1	1.9	43			
Mid	Post harvest	Primary P 75%	0-30	ZCL	24	2.6	26.6	86.7	2.86	0.154
			30-60	ZL	19.9	1.1	21.1			
			60-90	ZL	34.2	1.7	35.9			
Mid	Post harvest	Primary P 100%	0-30	ZCL	16.1	1.9	17.9	74.6	2.47	0.139
			30-60	ZL	23.4	3	26.4			
			60-90	ZL	49.7	6.5	56.2			
Mid	Post harvest	Primary P 125%	0-30	ZCL	25.9	3.6	29.5	67.5	2.38	0.145
			30-60	ZL	31.3	2.3	33.6			
			60-90	ZL	37.7	2.6	40.3			
Late	Pre drilling	Whole field	0-30	ZL	17.6	0.6	18.2	73.5	2.79	0.135
			30-60	ZL	27.3	0	27.3			
			60-90	ZCL	14.9	0	14.9			
Late	Post harvest	Untreated	0-30	ZCL	13.1	0.6	13.7	40.4	2.94	0.164
			30-60	ZCL	13.1	1	14.2			
			60-90	ZCL	14.4	0	14.4			
Late	Post harvest	Primary P 75%	0-30	ZCL	16	1.6	17.6	63	2.82	0.15
			30-60	ZCL	9.7	0	9.7			
			60-90	ZCL	6.7	0	6.7			
Late	Post harvest	Primary P 100%	0-30	ZCL	20	1.7	21.7	43.2	3.07	0.16
			30-60	ZCL	23	1.4	24.4			
			60-90	ZCL	9.9	0	9.9			
Late	Post harvest	Primary P 125%	0-30	ZCL	18.7	3	21.8	32.6	3.05	0.148
			30-60	ZCL	10.7	0.4	11.1			
			60-90	ZCL	6.3	0	6.3			

