1. PROJECT

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Title: Is there sufficient Ensifer and Rhizobium species diversity in UK farmland soils to support red clover (Trifolium pratense), white clover (T. repens) lucerne (Medicago sativa) and black medic (M. lupulina)?

Dates: 2011-2015

Funding organisations: Lawes Trust as well as the BBSRC Institute Strategic Programme Grant (ISPG), Optimisation of nutrients in soil-plant systems (BBS/E/C/0005196) at Rothamsted Research, the Research Endowment Trust Fund and School of Agriculture, Policy and Development at the University of Reading. Part of this study was also carried in association with the LegumeLINK project (LK09106) which was supported by the Sustainable Arable LINK programme by the Department for Environment Food and Rural Affairs (DEFRA), UK.

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2. DATASET

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Title: Quantification of nodulation in red clover (Trifolium pratense), white clover (T. repens), lucerne (Medicago sativa) and black medic (M. lupulina) in a range of 10 UK farm soils

Description: Soil was a collected from 10 organic farms across the UK and evaluated for the presence of compatible rhizobia for red and white clover, lucerne and black medic (aka yellow trefoil). The data details nodulation of plants growth in soil solutions from each farm, in triplicate, compared to control inoculum.

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Rights-holder(s): University of Reading and Rothamsted Research

Source(s): All sequences that have been used in the publication have been uploaded onto Genebank

3. TERMS OF USE

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4. CONTENTS

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Legume\_nodulation\_at\_10\_UK\_Farms.xlsx

5. METHOD and PROCESSING

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Ten UK sites were selected as part of the LegumeLINK project conducted from 2008-2011 (Döring et al, 2013\*).

Soil from each farm site composed soil which had previously received commercial rhizobium inoculum (‘ASM’), and control soil (uninoculated). Soil from each site was made into a soil suspension and used to inoculate four different legume species. The four plant species were also grown and inoculated with either the commercial inocula for 'clover' and 'lucerne' used in the original field trial (Legume Technology Ltd, Eastbridgford, UK) or with lab reference strains RCR221 and RCR2011.

The growing medium for inoculated plants was autoclaved fine vermiculite (1-3 mm, Sinclair, Gainsborough, UK), and involved placing 6 g into 50 ml Falcon tubes (Greiner Bio-one Ltd, Stonehouse, UK) with 20 ml sterile N-free nutrient solution containing 1 g CaPO4, 0.2 g K2HPO4, 0.2 g MgSO4.7H2O, 0.2 g NaCl and 0.1 g FeCl3 in 1 L water. Seeds were sterilised in 5 ml HOCl solution (Hypotech Ltd, Isle of Wight, UK) diluted to 600 ppm for 7 minutes (small seeds, <2 mm) or 15 minutes (large seeds, >2 mm) and rinsed 3 times in sterilised nanopure water. Several sterilised seeds were aseptically transferred to the tubes, which were then placed in a sealed zip-lock bag with a cotton wool bung to allow gas exchange. After one week, excess seedlings were removed, leaving one seedling per tube. Each seedling was inoculated with 1 ml of bacterial suspension. For experimental replicates, a soil suspension was made by mixing 10 g soil with 90 ml water, mixing for 2 minutes with a magnetic stirrer (Fisher Scientific UK Ltd, Loughborough, UK) at 300 rpm and adding 1 ml suspension per seedling using a wide-bore pipette. For positive controls 1 ml of overnight culture (OD600=1) was spun down in an Eppendorf Centrifuge 5415D (Eppendorf UK Ltd, Stevenage, UK) for 5 minutes at 5000 rpm, the supernatant removed, and cells re-suspended in 1 ml Phosphate Buffered Saline (PBS). PBS was prepared using pre-made tablets (Oxoid) dissolved in sterile distilled water. For negative controls 1 ml sterile PBS was added in place of a bacterial culture. The Rothamsted culture strains were: Rhizobium leguminosarum sv trifolii (strain no: RCR221, RCR226), Rhizobium leguminosarum sv viciae (strain no: RCR1001), Ensifer meliloti (strain no: RCR2011), Rhizobium gallicum sv gallicum (strain no: RCR3007), and Mesorhizobium loti (strain no: RCR3002, RCR3209).

In total 960 plants were grown, of which 720 were samples, and 240 negative controls. After 6 weeks of growth there was a mortality rate of 4.5%. Thus 917 plants were harvested, comprising 685 samples and 232 negative controls. After 6 weeks, plants were harvested. Plant height and above-ground biomass were measured. Root systems were washed in water to remove vermiculite, weighed, and nodules counted. The uppermost live root nodule was excised from the root system, and placed individually in a multi-well plate. Each nodule was sterilised in 70% ethanol for 2 minutes, and rinsed three times in NanopureTM-purified water (Thermo Fisher Scientific Biosciences GMBH, Altrincham, UK). Nodules were then crushed in 100 µl 40% glycerol using a hedgehog replica plater, and 20 µl of the resulting cell suspension streaked onto Yeast Malt Extract agar (per litre: Yeast extract, 1 g; Mannitol, 10 g; K2HPO4, 0.5 g; MgSO4, 0.2 g; NaCl, 0.1 g; Congo red dye, 0.025 g; Agar, 15 g; Ultra-pure water, 1 L) and grown for 48-72 hours at 27°C. Rhizobial colonies were identified by the non-uptake of the Congo red dye, secretion of extra-cellular polysaccharides (resulting in readily identifiable mucoid colonies) and non-fluorescence under UV light.

When uniform colony types were obtained, a single colony was aseptically streak plated onto tryptone yeast (TY) agar (per litre: Tryptone, 5 g; Yeast extract, 3 g; CaCl2.2H2O, 0.89 g; Agar, 17 g; Ultra-pure water, 1 L) to reduce inhibitory polysaccharide production; this ensured strain purification. Overnight cultures were made by aseptically transferring a single colony with a loop into 10 ml TY broth (recipe as before) and incubating at 27 °C on a rotary shaker for 24-72 hours at 200 rpm, until samples became turbid. Frozen stocks were made by combining 700 µl of overnight culture with 300 µl 40% glycerol in a cryovial. These were then stored at -80 °C until required.

All strains used in this study were either isolated from the root systems of legumes inoculated with soil from the participatory farm sites or accessed from the Rothamsted culture collection (Rothamsted Research, Harpenden, UK).

\* Döring, T.F., Baddeley, J.A., Brown, R., Collins, R., Crowley, O., Cuttle, S., Howlett, S.A., Jones, H.E., McCalman, H., Measures, M., Pearce, B.D., Pearce, H., Roderick, S., Stobart, R., Storkey, J., Tilston, E.L., Topp, K., Watson, C., Winkler, L.R., Wolfe, M.S., 2013. Project Report No. 513 Using legume-based mixtures to enhance the nitrogen use efficiency and economic viability of cropping systems. http://orgprints.org/24662/.