Plant Mechanisms Contributing to Acid Impairment of Nodulation of *Medicago murex* and *Medicago sativa* by *Sinorhizobium medicae*

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This thesis is presented for the degree of
Doctor of Philosophy
of
Murdoch University

2003

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Yvonne Cheng

Cause is effect concealed, and effect is cause revealed.

The Aghori Vimalananda

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PUBLICATIONS ARISING FROM THE THESIS

Cheng Y, Watkin ELJ, O'Hara GW, Howieson JG. 2002. *Medicago sativa* and *Medicago murex* differ in the nodulation response to soil acidity. *Plant and Soil* **238**: 31-39.

Cheng Y, Howieson JG, O'Hara GW, Watkin ELJ, Souche G, Jaillard B, Hinsinger P. 2003. Proton release by roots of *Medicago murex* and *Medicago sativa* growing in acidic conditions, and implications for rhizosphere pH changes and nodulation at low pH. Accepted for publication in *Soil Biology and Biochemistry*.

SUMMARY

The widespread sowing of the perennial forage legume *Medicago sativa* to lower groundwater tables in south-western Australia is limited as many soils targeted for its use are too acidic (pH_{CaCl_2} < 5) for consistent nodulation with *Sinorhizobium* spp. The annual medic *M. murex* grows and nodulates well in these acidic soils, but it cannot fill the niche of *M. sativa* in lowering groundwater tables. The differential ability of *M. murex* and *M. sativa* to nodulate in acid soils provided the opportunity to compare the nodulation responses between the two species and to identify the mechanisms contributing to the poor nodulation of *M. sativa* in soil of low pH.

An initial glasshouse experiment compared the nodulation of *M. murex* cv. Zodiac and *M. sativa* cv. Aquarius with *S. medicae* strains WSM419 and CC169. Subsequent glasshouse and laboratory experiments used only the more acid-tolerant *S. medicae* strain WSM419. In the glasshouse in soil of pH_{CaCl_2} 4.3, the uppermost nodule on both *M. murex* and *M. sativa* formed at 4-5 cm below the hypocotyl, but the nodules on *M. sativa* formed almost 4 weeks later than those on *M. murex*. The difference in nodulation response between *M. murex* and *M. sativa* was related to numbers of *S. medicae* in the rhizosphere. After 24 d growth in soil of pH_{CaCl_2} 4.3, there were 100-fold higher numbers of *S. medicae* WSM419 associated with the roots of *M. murex* than *M. sativa*. This difference in rhizobial numbers was not due to differences in root growth as there were similar rates of root elongation in *M. murex* and *M. sativa*, or differences in the root products released as root exudates of *M. murex* and *M. sativa* produced at low pH had no significant effect on the growth of *S. medicae*.

Using a 'root mat' approach on soil disks of pH_{CaCl_2} 4.49, *M. sativa* acidified its rhizosphere by approximately 0.2-0.4 pH-units within 4 d, while *M. murex* did not acidify its rhizosphere. Rates of H^+ release were higher from *M. sativa* than from *M. murex*. Using videodensitometry with agarose of pH 4.5, mature parts of the tap-root of both species exuded OH^- ions, but this was approximately 2-times higher in *M. murex* than in *M. sativa*. Consequently, young parts of the *M. sativa* rhizosphere were more acidic than that of *M. murex*. The higher rate of acidification by the roots of *M. sativa* made its rhizosphere less favourable for the survival and growth of *S. medicae*.

Root hair development was initially similar for both *M. murex* and *M. sativa*. However by 7 d after sowing in soil of pH_{CaCl_2} 4.3, the density of root hairs on *M. murex* increased to 37 root hairs mm^{-1} root, while the density of root hairs on *M. sativa* decreased to 20 root hairs mm^{-1} root. Due to higher root hair density, the roots of *M. murex* provided a greater surface area for the attachment and colonisation of *S. medicae* compared to the roots of *M. sativa*. Indeed, confocal laser scanning microscopy at 7 d after sowing showed there were larger populations of a green fluorescent protein-marked transconjugant of *S. medicae* WSM419 colonised at 4-5 cm below the hypocotyl on the root of *M. murex* (3.28 pixel intensity units) compared to *M. sativa* (1.78 pixel intensity units). The smaller population of *S. medicae* colonised on the *M. sativa* root resulted in the observed delay in nodule development in *M. sativa* compared to *M. murex*.

Two plant mechanisms contributed to the greater numbers of *S. medicae* in the *M. murex* rhizosphere compared to *M. sativa* rhizosphere when plants were grown in an acidic soil: (1) roots of *M. murex* had a higher density of root hairs, and thus provided a larger root surface area for the growth and

colonisation of *S. medicae* than *M. sativa*, and (2) roots of *M. murex* acidified the rhizosphere less, and thus provided more favourable conditions for the growth and colonisation of *S. medicae* than the rhizosphere of *M. sativa*.

Models explaining the different nodulation responses between *M. murex* and *M. sativa* in soil of pH_{CaCl_2} 4.3 and 7.0 are presented.

ACKNOWLEDGEMENTS

I acknowledge the financial support of the Australian Research Council by providing an Australian Postgraduate Award (Industry) scholarship. I sincerely thank my supervisors Assoc. Prof. John Howieson, Dr Graham O'Hara and Dr Elizabeth Watkin for sharing their knowledge and experience. Their encouragement and sense of direction kept me from losing my way.

The experiments described in Chapter 4 were done at the Institut National de la Recherche Agronomique, Montpellier, France, funded by a French Scientific Government Fellowship (French Embassy, Canberra, Australia), and a travel grant from the Australian Grains and Research Development Corporation. I am very grateful to Dr Philippe Hinsinger, Dr Benôit Jaillard and Gérard Souche for their supervision and support.

I acknowledge Dr Caixian Tang (LaTrobe University, formerly University of Western Australia) for his valuable discussion of the work in Chapter 4; Dr Ravi Tiwari (Centre for *Rhizobium* Studies, Murdoch University) for his advice in constructing the green fluorescent protein-marked *Sinorhizobium medicae* in Chapter 5; and Gordon Thomson (Murdoch University) and John Murphy (Centre for Microscopy and Microanalysis, University of Western Australia) for their assistance with microscopy described in Chapters 5 and 6.

I am grateful to my colleagues at the Centre of *Rhizobium* Studies, Murdoch University, and the Laboratoire de Science du Sol, Montpellier, for all their help, encouragement and friendship.

And to my family and friends, thank you for everything.

A NOTE ABOUT *SINORHIZOBIUM MELILOTI* AND *S. MEDICAE* IN THE THESIS

The rhizobial strains used in the experiments described in this thesis are referred to as *Sinorhizobium medicae* based on their 16 rDNA sequence and host nodulation (G Garau, pers. comm., March 2003). However, *S. meliloti* and *Rhizobium meliloti* described in previously published literature are referred to as *S. meliloti*.