Soils are Alive Newsletter

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Centre for Land Rehabilitation — The University of Western Australia

Welcome...

Lyn Abbott

This is the third issue of our *Soils are Alive* Newsletter. This time we take a look at rhizobia, which are the bacteria that convert nitrogen in the air into a form that plants can use. We also summarise some research on rhizobia conducted at The University of Western Australia.

Organic Agriculture

Organic agricultural practices aim to produce food that has a high nutritional value without the use of artificial fertilisers or synthetic chemicals such as pesticides. The objective is also to create sustainable farming systems. There are seven types of organic certification available in Australia. A new book called "The Organic Alternative" published by the Kondinin Group is a useful guide to organic farming in Australia (see *www.kondinin.com.au*).

The roles of soil organisms in organic agriculture is of great importance because these farming systems rely to a greater extent on cycling of nutrients from dead plant material in soil than do other forms of agriculture. Dr Richard Cookson has recently arrived at The University of Western Australia with support of a New Zealand Government Fellowship to investigate the fractions of organic matter that are important to soil organisms in organic agriculture. His research is important for understanding biological processes that occur in soil. Richard's email address is *wcookson@agric.uwa.edu.au*

Acknowledgements

This Newsletter was established with support from the Ian Potter Foundation which is also supporting the development of a website on soil health that will be available soon.

Ruth Morgan helped prepare this newsletter. Ruth, a Year 11 student at Penrhos College, was awarded a Faculty of Agriculture Scholarship at The University of Western Australia to work on soil biology and soil physics projects this summer.

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Rhizobia

by Krys Haq

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What are they?

Rhizobia are one of the best known groups of soil bacteria. They are extremely small, about one thousandth of a millimetre long, and though you're not likely to ever see them, you have probably seen the nodules they form on legume roots.

Acacia — Lupin

The shape and size of nodules are determined by the plant

How do nodules form?

Nodules form when specific chemical signals are exchanged between legume roots and rhizobia living in the soil. These signals allow the bacteria to recognise the root and enter it without being prevented by the plant's defence systems. Once inside the root, the bacteria trigger the plant cells to multiply many times to form a nodule. The bacteria change their function and wall structure and then start nitrogen fixation. The bacteria do not fix nitrogen when they live in soil with the rest of the microbial biomass.

Nitrogen fixation involves converting atmospheric nitrogen (N_2 gas) which plants cannot use, to ammonium (NH_4^+) which plants *can* use. It has been estimated that more than 90% of the nitrogen fixed by bacteria in nodules is rapidly made available to the plants.

The relationship between legumes and their root nodule bacteria is called a symbiosis because the plant and the bacteria live in a close, mutually beneficial association.

Efficient nitrogen fixation occurs in nodules that have a rich suply of a pigment called leghaemoglobin. This pigment is formed by the root and is like the haemoglobin in our blood. It supplies the nodule bacteria with the oxygen they need to fix nitrogen and keeps the oxygen away from the enzyme (nitrogenese) that is involved in the nitrogen fixing process. This means you can identify nodules that are fixing nitrogen effectively by the reddish pink colour inside the nodule when it's cut open.

Lupin Nodules

The pink colouring in the lupin nodule is due to the presence of leghaemoglobin. This is present when the nodules are fixing nitrogen.

A cut nodule can be seen in the figure below.

Rhizobia are a very diverse group of bacteria. You are probably aware that commercial inocula of different types of rhizobia are available to use on legume crop and pasture plants. Specific rhizobia have to be selected for each plant type.

Legumes grown in an area for the first time usually require the introduction of their appropriate rhizobia to grow successfully without the addition of nitrogen fertiliser.

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A B C

A: Field pea plus lupin bacteria- plants are yellow and nitrogen deficient
B: Field pea plus clover bacteria- plants are yellow and nitrogen deficient
C: Field pea plus pea bacteria- plants are green with no nitrogen deficiency
This soil was very deficient in nitrogen.

Even when the right type of rhizobia are present in soil and legumes are nodulated, the rates of nitrogen fixation may vary. This is because some of the bacteria fix more nitrogen than others. Therefore, the individual strains of bacteria that are chosed for large-scale commercial inoculum production need to be:

(1) bacteria that are most effective at fixing nitrogen and

(2) bacteria that survive best in the soil into which they are introduced.

Genetic diversity

Our studies have investigated the importance of genetic diversity amongst populations of rhizobia in clover, serradella and lupins in some filed sites in south-western Australia. These rhizobia belong to two distinct bacterial groups; *Rhizobium* (the root nodule bacteria of clover) and *Bradyrhizobium* (the root nodule bacteria of serradella and lupins). The earliest microbiological studies showed that these two groups of bacteria grow at different rates. The fast growing group was called *Rhizobium* and the slow growing group was called *Bradyrhizobium.*

Microbiological studies showed other differences between these groups of bacteria, but the full extent to which they differ only became clear when the new molecular biology technology was developed. Molecular comparisons of the DNA in rhizobia and bradyrhizobia showed that there are many differences between them.

Identifying diversity in root nodule bacteria

We investigated the diversity of rhizobia and bradyrhizobia in several pasture sites in Western Australia. We began our studies by preparing antibodies to the inoculant bacteria so that we could determine whether or not they were inside the nodules. To develop the specific tools for identification of the inoculant bacteria, bacterial cells from commercial inoculum of *Rhizobium* and the *Bradyrhizobium* were injected into separate rabbits. The rabbits produced antibodies against these bacteria (this is similar to what happens when we have a vaccination). The antibodies were collected from the rabbit's blood and joined to a dye so that they were visible under the microscope.

From agricultural fields, we isolated bacteria from clover and serradella collected from a variety of locations in south–western Australia, and mixed them with our antibody/dye preparations. The dye enabled us to identify whether the inoculan rhizobium were present.

In one experimental site near Albany, Western Australia, the inoculant strain was rarely present in nodules of subterranean clover. However, nodules on serradella plants were mostly occupied by the inoculant strain. The clover rhizobia were introduced many years previously and had apparently not survived at this site.

The antibody technique only allowed us to determine whether the commercial inoculant rhizobia were present. It did not give us any information about how many other similar types of rhizobia were present. Genetic diversity can be studied using DNA fingerprinting. For example, a technique called the Polymerase Chain Reaction (PCR) can be used. The PCR technique showed us that genetic diversity was different in each location.

DNA fingerprinting showed that the inoculant strain of clover rhizobia transferred some of its DNA to other strains even during one growing season. This exchange of DNA leads to greater diversity of rhizobia which might also correspond with differences in the capacity of the rhizobia to survive in soil or to fix nitrogen.

The important symbiosis formed between legumes and root nodule bacteria in agriculture contributes substantially to nitrogen in soil for agricultural production. The use of biologically fixed nitrogen greatly reduces the need for nitrogen fertiliser. This has been very important in developing the agriculture industry in many parts of Australia.

Workshops on soil biology

One day, two day and 12 day workshops on soil biology are run by the Centre for Land Rehabilitation at The University of Western Australia. The workshops introduce complex soil biological processes, emphasise the interrelationships between organisms and investigate what soil organisms look like, what they do and what kind of habitat they like to live in. This is provides the context for considering effects of land management practices on biological processes and selecting farming or horticulture practices that capture the benifits of origanisms in soil.

ERA Farming Company participants examine plants in their greenhouse experiment.

The 12 day workshop is run over 6 weekends spread throughout the year and includes a major experiment and analyses of soils and plants

One day workshop participants studing soil fauna.

One day workshops in country areas are coordinated by the Land Management Society.

Soil Biology Workshop Program 2001

Know Your Soil Biology, Soils are Alive
1 day at country venues on request
(minimum of 10 participants)
CONTACT: lmsinfo@space.net.au

Soil Biological Fertility Workshop (2 days)
at The University of Western Australia
18 -19th April 2001
CONTACT: labbott@cyllene.uwa.edu.au

Soil Biological Fertility Workshop (12 days- 6 weekends)
at The University of Western Australia
Commencing June 2001
CONTACT: labbott@cyllene.uwa.edu.au

The research on the genetic diversity of root nodule bacteria was conducted by Krys Haq, Alison McInnes and Margaret Collins with the support of:

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