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A WELL-NODULATED SOYA BEAN ROOT



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The story of a Southern Rhodesian achievement,
as recalled by its architect

In 1947, Ro and I, with our six-year-old daughter Susan, plunged hopefully into the post-war flood of immigration to Southern Rhodesia (Zimbabwe since 1979). On arrival, we were posted to “Grasslands” – a 7500-acre Estate near Marondera, serving as the country’s regional agricultural research station for the *high-rainfall-sandveld* – for me to take up my appointment as a Pasture Research Officer.

The Station’s dual mandate was, on the one hand, to improve methods of managing the grazing of the abundant natural grassland – the *veld* – and, on the other, to develop restorative pastures to rotate with the region’s exhaustive tobacco crop. However, the second task soon revealed an underlying need to get crops of any sort, including pastures, to thrive on the region’s hungry soils.

On assuming duty I was assigned to a veld-vegetation-survey. Among plants encountered was a tiny legume, *Crotalaria anthylllopsis*, the profusion of the nodulation of which so impressed me that I fell into the habit of inspecting the roots of any legume I came across. And thus was I attuned to all that followed (Corby, 1959, 1967, 1971, 1974, 1976, 1980, 1981, 1988, 1990, 1993).

The high-rainfall-sandveld of Southern Rhodesia – the country’s main cropping zone by reason of its rainfall and extent – comprises leached sands of daunting infertility. However, spectacular success in getting maize, the country’s staple cereal (chosen to pilot the search for the causes of the infertility), to flourish on such soils (Corby, 1957) was accompanied by persistent failure to get the legumes to do likewise. Eventually, this forced the conclusion that their associated bacteria must be at fault.

NITROGEN AND LIFE.. A feature of all living things is that their tissues contain proteins, all of which have a core of nitrogen. Most plants draw their nitrogen from the soil, while a few, notable among them the legumes, draw it also from the air. Animals, including Man, on the other hand, get it through their subsistence on other life.

NITROGEN AND NITROGEN-FIXATION. About four-fifths of Earth's atmosphere is nitrogen, there present in its elemental form.

Nitrogen is a sluggish element, stubbornly reluctant to combine with others, and requiring much energy to compel it to do so. In Nature, only a few microbes (and lightning) muster the requisite energy.

*Any process, be it natural or artificial, that combines nitrogen with another element is termed **nitrogen fixation**. Any such process is said to **fix** nitrogen, and the product is termed **fixed**, or **combined**, nitrogen.*

De-nitrifying soil-organisms reverse fixation, thus releasing nitrogen back to the atmosphere.

THE LEGUMES AND THEIR BACTERIA. There are about 19,000 species of legume, mostly tropical, but found wherever plants grow – Zimbabwe has some 530 species.

Among agricultural species are the numerous peas and beans, groundnuts, clovers, lucerne, lentils, and lupins. These all draw nitrogen into agriculture, while the edible-seeded species – the pulses – provide Man's diet with its main source of plant-protein.

*Most legumes have **nodules** – galls – on their roots (see frontispiece), which are formed by soil-bacteria known as **rhizobia**. Within the nodules these bacteria fix nitrogen, that is, convert it from the gaseous form, which most plants cannot use, to solid water-soluble forms, that all can use.*

Rhizobia are of differing sorts, such that, for example, those associating with the groundnut do not do so with the soya bean. And, within each sort are strains differing in their ability to fix nitrogen – some fixing much, and others, at the other extreme, very little. Specialised laboratories around the world seek out the most potent fixers for local agricultural use.

SOIL FERTILITY. With even the most fertile of soils, the limit to plant-growth is usually set by their nitrogen-content. Accordingly, it has long been customary to add nitrogenous substances to soils to increase their productivity. Among traditional nitrogenous additives are the excreta of grazing animals, farmyard-manure, guano, and natural deposits of nitre. Nowadays, however, man-made forms are

the commoner additive: among these are ammonia, ammonium nitrate, ammonium sulphate, calcium cyanamide, calcium nitrate, and urea. All of these are compounds of nitrogen, that is, with their nitrogen in the fixed form that plants can use.

In the practice of 'green-manuring' a crop, usually of a legume, is ploughed under as another means of increasing fertility.

NITROGEN IN CROPS AND PASTURES. When crops are harvested, practically all their nitrogen is removed from the site. If the crop be a nodulating legume, the nitrogen in its nodules will have been withdrawn to the ripening seed, and thus it too will be removed, to leave behind only their empty husks.

When pastures are grazed, some of their nitrogen will become body-tissue of the grazing animal, some will return to the pasture in the animal's excreta, and some, if the animal be lactating, will enter its milk.

Accordingly, in 1956, through the good offices of Raymond Staples, the Director of Agricultural Research, I was granted a fellowship by the Food & Agriculture Organisation of the United Nations. This I used in Australia, then leading research in the tropical rhizobial field, to learn to manipulate these remarkable organisms.

On returning home, I set up a laboratory and ancillary glass-house, with Wilhelm Sandmann in charge, to seek out the rhizobia best-suited to local use. Meanwhile, Rex Tattersfield, our plant-breeder, was creating varieties of soya bean for local use – work backed and eased by the new laboratory.

In 1961 we ventured to grow a whole field of soya bean, inoculating Rex's choice of variety with Wilhelm's choice of rhizobium. The resulting crop was triumphal, so much so that Charles Murray, the Secretary for Agriculture, asked me to set up a factory to make legume-inoculants for the farming industry.

LEGUME INOCULATION. It was known in Roman times that, if soil from an area where beans were growing well were sprinkled on an area where they were not, growth on the latter would improve. Once the reason for this – bacterial nitrogen-fixation within the nodules – had been discovered, in the 1880s, the invention of inoculants soon followed.

Inoculants are usually made by absorbing a broth teeming with rhizobia into a carrier of powdered peat (see Carriers below, and Glossary). This form is relatively easily and cheaply made, but it is bulky, impure, and does not keep well.

In the method of this account, moistened bagacillo (see Glossary) is sealed into a bag of plastic film, autoclaved, inoculated with rhizobia by injection, incubated, then cold-stored until used. This form is of minimal size, is a pure culture of rhizobia, and, cold-stored, keeps indefinitely. However, its manufacture requires strictly aseptic techniques, and it is, therefore, relatively expensive.

For use, inoculants are mixed with skimmed milk, or with water containing 5% of household sugar, and stirred into the seed about to be sown. The seed is then dried, thus gluing the rhizobia to it, and well-placing them to infect the emerging root as the sown seed germinates.

The Factory was opened a year later, with Barbara Elliot in charge. Down the years, she was succeeded in turn by Glynis McCrae, Danuta Plisco, Ann Holland, and Jean Grant.

At the outset, the Factory produced an agar-based inoculant in a flat-sided glass bottle (a ‘medicine-flat’). The pure cultures of rhizobia, central to making the inoculant, were provided by Wilhelm Sandmann, later by Mary Ryder (née Purdom), from the Laboratory’s Rhizobium Bank – prepared well away from the septic bustle of the Factory.

In 1965, to counter an international trade-embargo against Southern Rhodesia, Charles Murray asked me to gear the Factory to greater output. However, the inoculant contained imported materials, lack of any of which would have crippled the Factory. Accordingly, to lessen dependence on vulnerable imports, I set about combining the required expansion with a substitution of local materials, intending mainly to replace imported agar with local peat. A countrywide search yielded no true peat – no suitable material!

RHIZOBIAL CARRIERS. *Rhizobia, in common with other microbes, are manipulated in or on a carrier of some sort.*

In inoculants they have, historically, been carried in or on a variety of substances, notably peat or agar.

In the Marondera Laboratory they are carried on the surface of agar jelly, and, in the Factory, in an aqueous broth.

Next, we experimented with cob-earth as a possible carrier (Corby, 1976). It was found to be too variable in composition for reliable use in a factory routine!

Next, I flew to South Africa to explore the possibility of using palmeet, the peat of the Cape Town marshes, which was being used there as a carrier. Its delivered cost was prohibitive!

Then, visiting the Chiredzi sugar-mill, I chanced upon bagacillo, and sensed that I had found the elusive carrier.

Meanwhile, in 1967, I had moved to the Department of Botany of the University of Rhodesia, and there, funded by the Southern Rhodesia Government, helped by Winifred Petrie, and with Mary Ryder also helping at Marondera, we perfected the bagacillo-based inoculant, the technique of which was presented to the Factory in 1977.

INOCULANT CONTAINER. *The container for the inoculant is a bag of heavy-gauge high-density polythene film, which withstands both autoclaving and rough handling (see photograph on page 6). It is pervious to the respiratory gases, thus allowing the rhizobia to breathe, but almost impervious to water-vapour, thus long-maintaining a moist environment for them.*

Each bag suffices to inoculate the seed required to sow 1 hectare (2.5 acres) of crop.

Manufacture of the bagacillo inoculant was started in 1980. However, I had not appreciated that much work needed to be done to convert a successful laboratory technique into an equally successful factory routine. This failure bred multiplying problems, and, in



A BAG OF SOYA BEAN INOCULANT

1985, at the request of the Zimbabwe Government, and sponsored by the International Monetary Fund, I returned to Zimbabwe to make good the lapse (Corby, 1986) – we had retired to England in 1982.

The venture received helpful and encouraging endorsement. The Harare Farmers' Coöp gave One Thousand Guineas (some £34,000, or R400,000, today) towards the initial cost of equipping the Factory. When the Americans wound up their *Better Legume Inoculants* project, they gave us their collection of rhizobia. Dr Penelope Grant, Director of the nearby Soil Productivity Research Laboratory (established in 1963), was ever helpful.

The Factory's output peaked at some 130,000 packs a year in Year 2001. Demand was mainly for an inoculant for soya bean, the legume, above all others, with both commercial and dietary promise. Demand for it has been continuous, which suggests that soya bean rhizobia, asiatic in origin, do not persist in Rhodesian soils.

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To the succession of Factory Managers and their staff, all for maintaining the Factory's output in challenging circumstances;

To Wilhelm Sandmann, and, after him, Mary Ryder, for maintaining the Rhizobium Bank, and for providing cultures of rhizobia to the Factory; to Rex Tattersfield for breeding locally suitable soya beans; to the Department of Chemistry for searching for peat;

To Winifred Petrie and Mary Ryder for their help in developing the inoculant;

To the Americans for giving us their collection of rhizobia;

To Mary Ryder again, and to my daughter, Susan Vincent, for help in telling the story;

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REFERENCES

Corby HDL. 1957. Experiments in growing maize on sandveld soils. *Rhodesia Agricultural Journal* **54:** 129-172.

Corby HDL. 1959. The nutrition of legumes with particular reference to the high-rainfall sandveld of Southern Rhodesia. *Proceedings of the fourth conference of the professional officers of the Department of Research and Specialist Services of the Federal Ministry of Agriculture of Rhodesia and Nyasaland*. 24 pages. Government Printer, Salisbury.

Corby HDL. 1967. Progress with the legume bacteria in Rhodesia. *Proceedings of the Grassland Society of Southern Africa* **2:** 75-81.

Corby HDL. 1971. The shape of leguminous nodules and the colour of leguminous roots. In: Lie TA , Mulder EG, eds, Biological nitrogen fixation in natural and agricultural habitats. *Plant and Soil, Special Volume:* 305-314.

Corby HDL. 1974. Systematic implications of nodulation among Rhodesian legumes. *Kirkia* **9:** 301-329.

Corby HDL. 1976. A method of making a pure-culture, peat-type, legume-inoculant, using a substitute for peat. In: PS Nutman, ed. *International Biological Programme 7: Symbiotic Nitrogen Fixation in Plants:* 169-173. Cambridge University Press.

Corby HDL. 1980. Rhizobial root nodules in the classification of the Leguminosae. *Doctoral Thesis, Department of Botany, University of Rhodesia.* 171 pages. Library, University of Rhodesia.

Corby HDL. 1981. The systematic value of leguminous root nodules. In: Polhill RM, Raven PH, eds. *Advances in Legume Systematics, Part 2:* 657-669. Royal Botanic Gardens, Kew.

Corby HDL. 1986. *A report on a review of legume inoculant production at the Grasslands Research Station, Marondera, Zimbabwe. August-October, 1985.* 31 pages. Report to the AMEX Consultancy, Chelsea, London, as agents of the International Monetary Fund.

Corby HDL. 1988. Types of rhizobial nodules and their distribution among the Leguminosae. *Kirkia* **13:** 53-128.

Corby HDL. 1990. The incidence of rhizobial nodulation among legumes dominant in the *Flora Zambesiaca* area of Africa. *Kirkia* **13:** 365-375.

GLOSSARY

Agar: a derivative of seaweed. When suspended in water it forms a jelly, on the surface of which microbes may be grown and manipulated.

Autoclave: an apparatus for sterilization with super-heated steam.

Bagacillo: a fluffy by-product of sugar-cane-milling. Air-borne, it wafts into corners, there to accumulate in collectable quantities. It comprises scraps of sugar-cane-pith, with the following relevant properties: consisting of more or less pure cellulose, it has a constancy of behaviour invaluable to the routine of a factory process; consisting of torn cells, it abounds in nooks and crannies in which rhizobia may shelter; able to absorb as much as eleven times its own dry weight of water without becoming sodden, it provides an ideally moist environment for the rhizobia. However, as a result of changes in mill-routine, it's no longer available.

Bagasse: the dry residue of sugar-cane after the sugary sap has been squeezed from it.

Broth: a nutritified soup, suited, in this instance, to rhizobia.

Cob-earth derives from mouldering maize-cobs – to be found in a discarded heap on any maize-growing farm.

Peat: an accumulation of airlessly rotted marsh-vegetation. Rhodesian 'peat' is mostly sand.

POSTSCRIPT

Since my day, the Netherlands Government has funded a replacement of the original mud-brick Factory and Laboratory; the Oil Seeds Producers' Association has funded a replacement of the Factory's worn equipment; the International Monetary Fund provided Dr Paul Davis for a three-year stint as resident Consultant Microbiologist; bagacillo has been replaced by finely hammer-milled bagasse; the Factory has come under the wing of the Soil Productivity Research Laboratory, and is in charge of Joram Tapfuma.

The Factory, now half-a-century old, still flourishes, albeit hampered by electricity-power-cuts.

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Chombanji.

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