THE INTELLIGENT USE OF INOCULANTS AND LIME PELLETING FOR TROPICAL LEGUMES

D. O. NORRIS*

SUMMARY

The evolutionary history of the legume-*Rhizobium* symbiosis is described and it is pointed out that adaptation to acid soils and the possession of the slow-growing "cowpea" type of *Rhizobium* are primitive characteristics that have survived to the present day in the majority of tropical legumes. From this primitive type there have repeatedly developed specialized types with a high lime requirement and an acid-sensitive *Rhizobium*. It is for this type of legume, typified by clovers and medics, that the technique of lime pelleting the seed was developed. The reasons why this technique has little relevance to tropical pasture species are discussed, and practical guides to the need for liming the soil, the type of inoculum to use, and the type of pelleting material to employ are provided.

INTRODUCTION

Logical modification of traditional inoculation practice for tropical legumes requires that we seek a background, that is, an understanding of the origin and evolutionary history of the legume-*Rhizobium* symbiosis. The trouble with studies of this symbiosis since their inception late in the 19th century was that they were made by specialists interested in only one aspect of the subject. They were begun by agricultural chemists, followed by bacteriologists, followed by agronomists. The fact that a *symbiosis* was being studied received scant attention. Taxonomic botanists then, as now, ignored the presence of nodules on the roots of legumes; and it occurred to no-one that the evolutionary history of the legumes might have significance.

It was not until 1914 that the Australian E. C. Andrews (who was, incidentally, a geologist) considered the Leguminosae as a whole and pointed out that they had their origin under wet tropical conditions in upper Cretaceous times.

Their subsequent development has been one of adaptation to life under arid or temperate conditions, and may be stated in general terms as an outward migration into temperate zones. However, their origins are still clearly indicated in the modern distribution of species shown in Table 1 (based on Taubert's treatment in Engler and Prantl, 1894).

TABLE 1.
World distribution of Leguminosae (After Taubert, 1894)

Number of Genera	Number of Species	Genera and Species in Tropics and Sub-Tropics	Genera and Species in Temperate Regions
Caesalpiniaceae			
95	1032	89- 988	7- 44
Mimosaceae			
31	1341	31-1200	1- 141
Papilionaceae			
305	6514	176-2430	141-4084
Grand Total all Leguminosae			
431	8887	296-4618	149-4269

It is currently estimated that there are about 14,000 species in Leguminosae but the general picture does not differ from that shown in Taubert's figures. Tutin (1958) provides a good outline of the origin and classification of the legumes which should be read by every student of *Rhizobium*.

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* C.S.I.R.O., Division of Tropical Pastures, St. Lucia, Brisbane, Queensland 4067

The original basic form of the legumes, which is retained by a large number of tropical species today, was that of a large soft-leaved, evergreen tree in the rain-forest. This growth form is only possible in a mild, unvarying, perennially moist environment so that in the course of their invasion of arid, sub-tropical or temperate regions the legumes have had to change their form in response to their changed environment. The general course of this change has been from large woody trees → shrubs and woody lianes → perennial herbs → annual herbs. On the basis of growth form alone the common annual herbaceous legumes of temperate agriculture, the peas, beans, vetches, clovers and medics are highly evolved species. So are some tropicals, e.g. Townsville lucerne, but in the case of the tropicals specialization of growth form has been made without sacrificing the benefits of the primitive type of symbiosis, as will be discussed below.

THE ORIGIN OF THE SYMBIOSIS

It was not until 1956 that the mass of data about *Rhizobium* that had been accumulating for more than half a century was reviewed in relation to the evolutionary history of the symbiosis. I was led to do this (Norris, 1956) by being placed in a tropical environment where it became obvious that many of the current views on *Rhizobium*, based on studies with clover, did not agree with common observation. In particular, possession of the slow-growing, widely cross-inoculating "cowpea" type of *Rhizobium* by a large number of tropical species had to be accounted for.

The following points were assembled and considered —

(a) Since the legumes arose in, and evolved away from, the wet tropics, and most of those that remain there today, including the more primitive types, are nodulated, the symbiosis must also have arisen under wet tropical or rainforest conditions. Rainforest soils are characteristically very acid and poor in nutrients including calcium.

(b) Cross-inoculation promiscuity (i.e. ability to nodulate effectively with a very wide range of bacterial strains) represents a primitive condition. It was shown by Wilson (1959) that there is a positive correlation between promiscuity and the open pollinated condition which is primitive. Without promiscuity in the early days of the symbiosis it could not have survived in the absence of seed transmission.

(c) The modern "cowpea" *Rhizobium* is perfectly at home under rainforest conditions. It can be isolated from almost any legume one cares to choose from this environment. Furthermore, species having this type of *Rhizobium* are well known to be promiscuous, e.g. the cowpea.

(d) The "cowpea" type of *Rhizobium* is common to the majority of tropical species covering each of the great sub-divisions of Leguminosae, and therefore must pre-date the division of Leguminosae into its three sections Caesalpiniaceae, Mimosaceae and Papilionaceae.

The conclusion is inescapable that the slow-growing "cowpea" type of *Rhizobium* represents a survival of the ancestral type, and all other types of *Rhizobium* such as the fast-growing clover type must be advanced derivatives.

Acceptance of the view that the basic condition of the symbiosis was non-specificity (or promiscuity) from which specificity has progressively developed clarifies the business of the Cross-inoculation Groups. A Cross-inoculation Group is a group of botanically related species of legume which will nodulate effectively with each other's *Rhizobium* but not with that of other less related species. Thus, we have a Clover Cross-inoculation Group, a Lotus Cross-inoculation Group and so on. It came to be believed that every legume should belong to some group or other. Any which showed ability to cross-inoculate with cowpea were pushed into the Cowpea Cross-inoculation Group. These groups are the "sacred cows" of legume bacteriology which have lain across the path of our understanding of legume-*Rhizobium* relationships for many years. They have been and still are revered by true believers in the "normality" of the symbiotic relationships in such groups of species as the clovers and medics, but the time has come when they must give way to modern traffic. If we are prepared to think about the subject it is easily understood that as a legume develops physiological specialization during evolution away from the ancestral tropical type it will become selective for its bacterial partner, since their relationship is a question of delicate biochemical balance. If a group of legume species evolves fast and forms a physiologically closely related "species cluster" its members will share the ability to symbiose with a special type of *Rhizobium* and will form a "Cross-inoculation Group". If a legume is not part of a species cluster but becomes nonetheless physiologically specialized for its *Rhizobium*, it does not fit into any cross-inoculation group but becomes one of those that we are accustomed to call "strain specific", i.e. one of the innumerable "exceptions" to the old cross-inoculation groups. The old "Cowpea Cross-inoculation Group" is revealed to be a myth. It is simply the great assemblage of all those legumes (the majority) which retain the primitive symbiotically unspecialized condition, and the ancestral cowpea type of *Rhizobium*. By no stretch of imagination can it be called a "group" in the sense implied by cross-inoculation grouping.

This view of cross-inoculation grouping makes it clear how there can be two quite distinct clover cross-inoculation groups, the old one embracing the common European clovers and another embracing an isolated group of species inhabiting the mountains of East Africa (Norris 1959a, Norris and 't Mannetje, 1964), and how numerous sub-groupings of compatible species can be discerned even within a classical cross-inoculation group such as the *Medicago* group (Brockwell and Hely, 1966). Indeed it would be strange if such were not the case. The processes of speciation and geographical isolation make it inevitable.

With this systematized view of cross-inoculation relationships we can also turn the subject of Cross-inoculation Groups from a morass of confused thinking and apparently unlimited "exceptions" into a useful supplementary tool for determining the phylogenetic position of species within specialized groups. The method for lack of a better term has been called "Symbio-taxonomy" (Norris, 1965a) and it depends on systematic reciprocal cross-inoculation with a spectrum of strains known to be fully effective on each homologous host; and careful recording of whether the result is effective nodulation, ineffective nodulation or failure to nodulate. The functioning of this method depends on the fact that as species diverge the ability to symbiose effectively with each other's *Rhizobium* is lost before the ability to form nodules. Effectiveness is lost before infectivity.

CALCIUM NUTRITION OF PLANT AND BACTERIUM

Having established the tropical origin of the symbiosis, it was a natural step to re-examine the question of the relationship of calcium to the formation of nodules and to the growth of the bacteria. Studies with clover and lucerne had led to the conventional view that abundance of lime was necessary for successful nodulation, both because it was necessary for nodule formation and because the bacteria were sensitive to calcium and needed large quantities of it for growth. In spite of research evidence to the contrary, many laboratories still add large quantities of calcium carbonate to culture media for growth of *Rhizobium*. Whereas there could be little doubt, in the face of abundant practical experience, that lime improves nodulation of such species as clovers on acid soils, there was room for considerable scepticism about its value for tropical species. In a tropical environment, particularly in rain-forest situations, legumes can be observed on all sides copiously nodulating on soils of extreme acidity. Is this because the host legume does not need calcium for nodulation, is it efficient at getting its calcium, does the associated *Rhizobium* perhaps need no calcium? It was decided to check the final point first and an extensive examination was made of *Rhizobium* growth under calcium — deprived conditions, utilizing in all 96 strains of all types (Norris, 1959b). The conclusion was that the calcium need of *Rhizobium*, if any, must be so small that it is capable of satisfaction from trace impurities in the growth medium. *Rhizobium* was, however, shown to be very sensitive to magnesium. Subsequent careful quantitative work by Bergersen (1961) and Vincent (1962) established that *Rhizobium* does have a calcium requirement which is about one-eighth of its magnesium requirement. The amount involved (a maximum of about 1 p.p.m.) is far too small to have any possible limiting effect on *Rhizobium* performance in the field for the simple reason that the legume host would fail from calcium deficiency long before the bacteria were affected.

The role of calcium supply in the formation of nodules and their proper functioning is, however, a very different matter. This is an effect on the host legume, not on the bacteria. Abundant evidence testifies the importance of ample calcium supply in this aspect of the symbiosis (e.g. Loneragan, 1959) where such species as clovers and lucerne are concerned. Without adequate calcium nodules do not form, and in those that do form the fixation of nitrogen is greatly reduced. When, however, we look around in a tropical or sub-tropical environment it is not hard to find instances where legumes are nodulating in soils where calcium supply is precarious. The Wallum soils of Queensland are a very good example. On these highly infertile soils the calcium content varies from 0.1 to 1.6 m.e. %. In pot studies with such soils lucerne, if not given lime dressings, will die from acute calcium deficiency in the early seedling stages. Yet on these soils there flourishes a high population of well-nodulated legumes in such genera as *Acacia, Pultenaea, Daviesia* and *Gompholobium*. All of these have cowpea type *Rhizobium* which will cross-inoculate with such promiscuous species as the cowpea, and are obviously able to obtain all the calcium that they need. How do they do it? Since the Ca levels in their tissues are not extremely low, there is only one way that they can do it — they have developed great efficiency at collecting and assimilating their calcium from the soil. That the same efficient mechanism is possessed by some of the herbaceous tropical pasture legumes was indicated in studies made by Andrew and Norris (1961) in which *Desmodium uncinatum, Stylosanthes gracilis, Indigofera spicata, Phaseolus lathyroides* and *Centrosema pubescens* were grown on an extremely Ca-deficient Wallum soil at various levels of calcium amendment, and compared with the common temperate legumes white clover, lucerne, barrel medic and strawberry clover.

Although on this soil of very low exchange capacity it takes only small amendments of lime to reach a degree of saturation with Ca at which even the temperate species can operate, the results left little doubt that at the extreme deficiency levels the tropicals as a group were greatly superior to the temperates at etxracting their calcium needs. It was also clearly indicated, as would indeed be expected, that a range of efficiency existed. One of the tropicals, *Desmodium uncinatum*, was in fact very little superior to white clover at getting its calcium. White clover, however, was very much more efficient than other temperate species. It is probable that this high efficiency of white clover underlies its ability to grow in many unlikely places. It is a polymorphic species and some of its races have been shown by such studies as those of Snaydon (1962) to possess quite high tolerance of acid soil conditions and their attendant problems such as low calcium and high manganese.

ACID PRODUCTION BY RHIZOBIUM

Rhizobium when grown in culture develops either an acid or an alkaline reaction or always an alkaline reaction when tried on a wide range of culture media, reaction in the culture medium. A given strain may not produce always an acid but the general overall result enables definite classification as an acid or alkali producer. This effect can be clearly seen in tabulated data such as those of Walker and Brown (1930) or Galli (1959). It has recently been suggested (Norris, 1956b) that this sub-division of *Rhizobium* is of basic significance.

The slow-growing alkali-producing habit in *Rhizobium* is the original primitive condition, still found in most tropical legumes as long as they continue to inhabit acid soils similar to those favoured by their ancestors. It is a stable persistent condition because it confers survival value in an acid soil if the organism during its multiplication in the rhizosphere can help to neutralize the acidity of its surroundings. Mutation to acid production has negative survival value. When, however, the host plant adopts alkaline soils for its permanent habitat selection pressure on the Rhizobium is reversed. Acid production is no longer inimical to survival, because the alkaline soil can take care of any acid produced. Moreover, the acid-producing habit is associated with faster growth rate and greater competitive power against other rhizosphere micro-organisms. Repeatedly therefore adoption by the host of alkaline soils has resulted in the development of a fast-growing acid-producing *Rhizobium.* We see this most strongly in the case of clovers and medics of the Tribe Trifolieae and peas and vetches of the Tribe Vicieae. In these instances the whole tribe is adapted, but in other tribes of Leguminosae, only certain genera or even parts of genera are adapted and accompanied by acid-producing *Rhizobium.* At this moment no instance of adaption is known within the Caesalpiniaceae, but within Mimosaceae the genera *Leucaena* and *Neptunia* are adapted. Within Papilionaceae, in addition to the Tribes Trifolieae and Vicieae mentioned above, adaptation is known to have occurred in *Astragalus, Psoralea* and *Sesbania* of Tribe Galegeae, in *Adesmia* and *Hedysarum* of Tribe Hedysareae, in part of the genus *Lotus* in Tribe Loteae, and in part of the genus *Phaseolus (P. vulgaris* and *P. coccineus)* of Tribe Phaseoleae.

It is important to remember the apparent contradiction of terms in this matter: Alkali-producing *Rhizobium* strains are associated with legumes adapted to acid soils; acid-producing *Rhizobium* strains with legumes adapted to alkaline soils. Recognition of the category to which any particular strain belongs is an easy matter. The strain is cultured for a month on a standard yeast-extract mannitol medium containing brom-thymol blue pH indicator. If the strain is an alkali-producer the medium turns blue, if an acid-producer it turns yellow. This simple test can provide invaluable information to plant introduction officers concerned with new legumes. If such an examination of even a small number of effective strains from a new legume indicates that they are acid-producers it is a strong indication that the species has specialized soil requirements and will probably respond to lime additions on acid soils.

As well as the simple question of whether a *Rhizobium* strain is an acid or an alkali producer there is much to be gained from a consideration of the relative acid producing power of strains within an adapted complex such as the clover complex. It is to be expected that *Rhizobium* strains from clovers growing on acid soils will be much weaker acid producers than those strains from clovers growing on alkaline soils. A highly significant correlation of this type has already been shown by Brockwell *et al.* (1966) after an examination of many clover and lotus strains. It follows from this that if we wish to push clovers on to acid soils we should deliberately select as inoculants those *Rhizobium* strains showing minimal acid production, instead of blindly using strains selected solely on nitrogen fixing ability as has been customary in the past. Work along these lines has already begun in both Australia and New Zealand.

THE BEARING OF ALL THIS ON INTELLIGENT USE OF INOCULANTS

The Question of the Right Strain of Rhizobium for Inoculation

Since the development of strain specificity by a legume is the direct result of specialized evolution, it follows that the taxonomic position of the legume is all-important when searching for a *Rhizobium* strain to use as an inoculant. The old idea that all legumes could be fitted nicely into one or other cross-inoculation group is no longer tenable. When a strain of *Rhizobium* is sought for a new legume there is really no substitute for conducting tests with a number of strains isolated from that species itself in the first place. If these are lacking then strains from its nearest botanical relation should be tried.

Common names of legumes are quite misleading in this context. To quote but one example, if we were to inoculate "Townsville lucerne" with culture prepared for ordinary lucerne it would be a waste of time and money for they are quite unrelated botanically. Townsville lucerne *(Stylosanthes humilis)* is in the Tribe Hedysareae and uses cowpea type *Rhizobium*, ordinary lucerne *(Medicago sativa)* is in the Tribe Trifolieae and has a highly specialized Rhizobium that will nodulate only with *Medicago, Melilotus* and *Trigonella* spp.

Much of the legume plant introduction work done prior to 15 years ago, in so far as it concerns species that were discarded because of lack of vigour, is suspect because of lack of attention to specific *Rhizobium* requirements. In many cases a satisfactory inoculant for a new species can be found amongst strains already present in the country because the legume species has not developed marked strain specificity. In other cases we must go back to the country of origin to collect the specific *Rhizobium*. This had to be done for *Lotononis bainesii* for which a highly specific and peculiar red *Rhizobium* was obtained (Norris, 1958) from South Africa. This introduction made the difference between complete failure in early trials and the commercial success of the species as a pasture plant at the present time. Interest thus stimulated in the genus *Lotononis* led to the introduction of *Lotononis angolensis* which refused to co-operate with the *Lotononis bainesii Rhizobium*. Further collecting in Central Africa had to be done to obtain another peculiar pink *Rhizobium* for *L. angolensis*, which now in its turn is becoming of increasing interest as a pasture species. Collection in Central Africa also had to be done before we could equip the African *Trifolium* species with effective inoculants, because they will not fix nitrogen with the inoculants prepared for common clovers.

Work with this type of legume is on an "all-or-nothing" basis. Unless you inoculate them you waste your time planting them. Other species of legumes, however, may possess strain specificity of an intermediate type. If not inoculated they will select a low population of effective bacteria from amongst the native *Rhizobium* already in the soil and establish in a slow and patchy fashion which finally builds up to a successful establishment, perhaps in the second year. In this category we find *Desmodium* spp., *Glycine javanica, Dolichos lab lab,* and *Centrosema pubescens*. Inoculation gives great advantage in uniformity and speed of establishment.

In brief, one should never make assumptions about what inoculant to use for any legume species. Unless advice is received from the seed supplier the opinion of a legume bacteriologist should be sought. He too may not know, but he is usually in a position to make an informed guess.

The impact of this on legume breeding is important. The plant dominates the symbiosis and its ability to accept or reject the bacterium is a hereditary character. In nature a non-nodulating or shy-nodulating habit is recessive because it functions virtually as a lethal factor. When man begins to recombine characters these recessives are likely to come to the surface, and types appear that are perhaps agronomically desirable, but are a problem to the legume bacteriologist. He has then to seek specific bacterial strains that will work with the new legume variety and incorporate them in inoculants with a consequent increase in cost and trouble. The dangers of this have been underlined by Gibson (1962) who showed that the lucerne variety Rambler will not work effectively with any of the common lucerne inoculants used in Australia. The more intensively a species has been bred by man the more complex is the inoculant situation. Soybeans are an outstanding example. In Table 2 is summarised the result of a glasshouse trial, using the Leonard jar technique, with nine strains of *Rhizobium* on five varieties of soybean commonly grown in Queensland.

TABLE 2.

Soybean: Dry matter yields per jar expressed as % of best for each of 5 varieties. Figures are means of 3 replicates of 2 plants, 54 days after planting, 42 days after inoculation.

Rhizobium Strain	Soybean Variety					Cumulative % score (Maximum = 500)
	M.B.H.	Bourke	Wills	Leslie	Nanda	
CB1003	68	67	46	53	60	294
CB1752	68	83	87	62	80	380
CB1786	84	83	89	61	84	401
CB1791	80	72	80	58	81	371
CB1795	82	100	80	63	100	425
CB1802	93	74	100	75	97	439
CB1809	100	99	95	100	95	489
CB1911	83	90	76	58	73	380
CB1927	86	92	74	66	82	400
Not inoculated	47	42	43	34	47	213

Even though these strains were pre-selected from a large range of strains for ability to fix nitrogen on these varieties it can be seen that their performance is very variable from variety to variety, and only one bacterial strain, CB1809, can be selected for outstanding performance on all five varieties. If we were to try five more varieties from a different source an entirely different pattern of response would result. Every new variety must first be screened against the old inoculants before any meaningful yield experiments can be embarked upon.

Most of the tropical legumes at present in use for pasture purposes are still pretty much in the condition in which nature bestowed them and plant breeders have only recently begun their adaptation to local requirement. Nevertheless, the seeds of future trouble are there and need to be avoided. Bowen and Kennedy (1961) demonstrated the existence of a hereditary shy-nodulating character in unselected imported seed of *Centrosema pubescens*, and it is well known to all who work with *Glycine javanica* that it is a poor and tardy nodulator. Unless carefully watched, both of these species might easily give rise to types that possess fine agronomic features but are virtually useless because of deficient nitrogen production.

The Question of Liming the Soil

In view of our realization that tropical species of legume possessing the alkali-producing type of *Rhizobium* are adapted to acid soils and efficient at obtaining their calcium needs for nodulation, we should always question the need to add lime to the soil to grow them. "Lime for legumes" is a saying that originated with and applies primarily to the specialized clovers, medics, peas and vetches. For most of our tropical legume species the saying should be "Lime for legumes — perhaps, in small quantities, and only if the soil is very acid or manganese-toxic or actually deficient in calcium". With these species the need for lime is a matter for the plant nutritionist not the rule-of-thumb man. Yield responses to lime by tropical species like Siratro *(Phaseolus atropurpureus)* undoubtedly have been and will be obtained on occasion. They are not nodulation responses but are growth responses to limiting nutrients made available by the lime and if we can determine what these limiting nutrients are and add them the need for the lime disappears. This effect is clearly seen in the results of Landrau, Samuels and Rodriguez (1953) with tropical kudzu *(Pueraria phaseoloides).*

However, as with all biological phenomena, no hard and fast line of division can be laid down in this matter between 'tropicals" and "temperates". Amongst the temperates we have the clovers, medics, peas and vetches which are lime-loving, but we also have species like the lupins, many of which have slow-growing cowpea type *Rhizobium*, grow on acid soils and are definitely intolerant of lime. This applies also to *Lotononis bainesii* which is frequently lumped with the "tropicals" but originates in fact from a warm temperate region in South Africa and has a considerable degree of cold resistance. It is adapted to acid sandy soils and its *Rhizobium* reflects this in being one of the strongest alkali-producers. In sand culture in Brisbane it has nodulated and grown quite successfully at pH 4.0 and in the presence of only 5 p.p.m. calcium (Andrew and Norris, unpublished). Lime is certainly not required by this species.

Conversely amongst the "tropicals" there occur groups in which the same process that we see in the clovers and medics has gone on. Adaptation of the host to better type soils has led to specialization of the *Rhizobium.* At the moment the only outstanding example of this amongst species in pastoral use is *Leucaena leucocephala.* This species in its native habitat in the Caribbean flourishes on coral benches and beaches. Elsewhere in its now worldwide distribution it flourishes on volcanic soils of high base status as in Hawaii or on limestone areas. Its *Rhizobium* reflects this in being a fast-growing acid-producer very similar to clover *Rhizobium*, and is highly specific. Selection of soils of high base status for *Leucaena* is clearly indicated and a nodulation response to lime on poor soils would be expected.

Amongst other "tropicals" there occur borderline cases in which no overall change in type of *Rhizobium* has occurred, but the host species appears to have inferior extracting power for calcium, which in turn may lead to poor nodulation, although outright failure of nodulation is unlikely except under extreme conditions. Two such species are *Desmodium uncinatum* and *Glycine javanica.* The former was found by Andrew and Norris (1961) to be relatively inefficient at obtaining its calcium compared with species such as *Stylosanthes gracilis.* In further work (Andrew and Norris, unpublished). *Glycine javanica* (var. Cooper) has shown similar behaviour. Below pH 5.5, nodulation begins to suffer and improvement from calcium addition occurs, even though the same *Rhizobium* strain is being used that simultaneously produces quite satisfactory nodulation on a host such as *Stylosanthes humilis* at pH 4.5, i.e. the effect is on the host's calcium metabolism, not on *Rhizobium* survival. This characteristic of *Glycine javanica* is responsible for many conflicting reports of lime response with this species in the field. It can be appreciated that on some soils a heavy application of superphosphate would provide adequate calcium for nodulation, on others where little superphosphate was added perhaps a lime response might be obtained.

An attempt to provide a guide to both *Rhizobium* specificity and lime sensitivity for the species which are currently in use in Australian tropical pasture work is made in Table 3. In this table the inoculum requirement is indicated as a group inoculant or as a specific. Thus, white clover is satisfactorily served by the clover inoculant prepared for all common clovers, but Kenya white clover requires a specific strain and the common clover inoculant is useless on it.

TABLE 3.
A guide to inoculum and lime requirement of legumes used in tropical pastures.

Species	Common Name	Expected Lime Response	Inoculum Requirement
Calopogonium mucunoides	Calopo	No	Cowpea*
Centrosema pubescens	Centro	No	Specific
Desmodium intortum	Greenleaf Desmodium	No	Desmodium
Desmodium uncinatum	Silverleaf Desmodium	Rarely, in extreme conditions	Desmodium
Dolichos axillaris	Archer dolichos	No	Cowpea*
Dolichos biflorus	Leichardt dolichos	No	Cowpea*
Dolichos lab lab	Rongai dolichos	No	Cowpea
Glycine javanica	Cooper, Clarence or Tinaroo glycine	Occasionally at pH below 5.5	Cowpea
Leucaena leucocephala	Peruvian leucaena	Yes	Specific
Lotononis bainesii	Miles lotononis	No	Specific
Medicago sativa	Lucerne	Yes, lime is obligatory if pH is 5.5 or lower	Lucerne
Phaseolus atropurpureus	Siratro	No	Cowpea*
Phaseolus aureus	Golden gram	No	Cowpea*
Phaseolus lathyroides	Phasey bean	No	Cowpea*
Phaseolus mungo	Mung bean	No	Cowpea*
Pueraria phaseoloides	Tropical kudzu	No	Cowpea*
Stylosanthes gracilis	Schofield stylo	No	Cowpea*
Stylosanthes gracilis	Oxley fine stem stylo	No	Specific
Stylosanthes humilis	Townsville lucerne	No	Cowpea*
Trifolium repens	White clover	Yes	Clover
Trifolium semipilosum	Kenya white clover	Yes	Specific
Vigna luteola	Dalrymple vigna	No	Cowpea*
Vigna sinensis	Cowpea	No	Cowpea*

* indicates a promiscuous species which will normally nodulate from native cowpea *Rhizobium* even if not inoculated.

The Question of Lime-pelleting the Seed

The technique of lime-pelleting was developed in Australia to overcome problems of nodulation failure in subterranean clover in attempts to develop pasture on marginal, acid, low-calcium soils (Loneragan *et al.* 1955; Brockwell, 1963). By wrapping the seed, after inoculation, in a coating of calcium carbonate stuck on with a biologically harmless adhesive such as 45% gum arabic or 5% Cellofas A (methyl ethyl cellulose) or 5% pure methyl cellulose, the acid-sensitive bacteria are protected from the acid soil and also a supply of calcium that may be needed for nodulation is provided immediately around the germinating seed. Good nodulation may be induced in this way by a very small amount of lime equal to about half the weight of the seed sown, which would otherwise require quite large amounts applied as a soil dressing.

In New Zealand where pelleting has been enthusiastically adopted, the procedure has been modified by the use of a 50/50 mixture of dolomite and Gafsa rock phosphate as a coating, which gives better nodulation than lime alone (Hastings and Drake, 1960).

In specific problem areas of southern New South Wales requiring aerial sowing, pelleting has been brought to its highest pitch with the introduction of the "Three Step Process" by F. Hely of C.S.I.R.O., Canberra (Hely, 1965). In this process vastly increased numbers of bacteria (700,000 - 800,000 per seed) are stuck on by first allowing the seed to soak up a bacterial broth, then adding a pellet incorporating a heavy inoculation with peat culture, and finally giving an outer coat of clay + lime + gum arabic to make a tough pellet. This process is designed to overcome by sheer weight of numbers excessive mortality of the bacteria in the period between air drop and the successful germination of the seed, which may be several weeks later.

While the primary purpose of lime pelleting was to overcome soil acidity problems, it was soon realized that the technique had a highly important secondary function. It provided an efficient means of "pre-inoculation" in the case of clover and medic sowings. Providing peat culture is used the bacteria inside the pellet are protected and the inoculated seed can be held for a considerable time before sowing. Hastings and Drake (1960) had excellent nodulation of white clover after holding pelleted seed for 92 days. Brockwell (1962) showed similar results with sub clover, Murguia and Date (1965) with white and sub clover, Brockwell and Whalley (1962) with barrel medic, Goss and Shipton (1965) with sub clover and barrel medic. In the latter two cases the inoculum also resisted sowing into dry soil several weeks before rain.

A tertiary function of the lime pellet is that its presence allows inoculated seed to be mixed with superphosphate for sowing, without killing the inoculant. This practice was taboo in the old days because the high acidity of superphosphate was quickly lethal to the bacteria on the seed surface when they were not protected by the neutralizing action of the lime coat.

In view of these successes it seemed to many that lime-pelleting was the answer to all our inoculant problems, and a vista of mutually beneficial sales of pre-inoculated lime-pelleted seed opened up to seedsmen and farmers. Over-hasty official recommendations have been made that all legume seed, regardless of species, should be lime-pelleted before sowing.

The most telling argument against universal lime-pelleting of legume seed by seedsmen for sale to the farmer is a financial one. Since up to 60% by weight of lime is added to the seed during pelleting, the farmer usually pays a very fancy price for this lime when he buys pre-pelleted seed, even if he is charged the same price for pelleted seed as for plain seed. To justify this extra cost it is imperative that he be guaranteed in return some real benefit in the form of outstandingly improved establishment and performance of his legumes. In the case of tropical legumes this guarantee can not be given at the present time. All spectacular successes with lime-pelleting and pre-inoculation have been with fast-growing acid-producing *Rhizobium* and legume species like lucerne and clover. This has little relevance to the majority of sowing of tropical legumes where we are dealing with species naturally adapted to acid soils, efficient at obtaining their calcium for nodulation, and possessing acid-tolerant (alkali-producing) *Rhizobium*.

Work published to date provides little support for the lime-pelleting of species with this type of *Rhizobium*. Parker and Oakley (1966) working with *Lupinus luteus* (yellow lupin), *Ornithopus sativus* and *O. compressus* (serradella) in West Australia, and using agar cultures consistently obtained significant *depression* of nodulation by lime pellet at several sites and in several seasons. In an attempt to demonstrate that the cause of this trouble was the agar cultures Shipton and Parker (1967) conducted further extensive trials in which peat and agar cultures were compared, with and without lime-pelleting. In the case of yellow lupin the agar cultures showed a significant depression of nodulation by lime pelleting in three out of four tests and no change in the fourth test. With peat cultures lime-pelleting increased nodulation in one test and depressed nodulation in two tests, but differences were not statistically significant. In the case of serradella, the agar cultures showed a depression of nodulation by lime-pelleting in six out of seven tests (three of them statistically significant) and an insignificant increase in the seventh test. The peat cultures showed a significant reduction of nodulation by lime-pelleting in two out of four tests, a significant increase in one test and no change in the fourth test.

Cloonan and Vincent (1967) used lime pelleting in association with peat culture in an attempt to improve nodulation of *Dolichos lab lab* and cowpea on red basalt soils of pH 5.0 - 5.5 in the Lismore area of New South Wales. *Dolichos* was tested at four sites, with cowpea also at two of them. At only one site did lime-pelleting produce significantly more nodules than ordinary inoculation with peat slurry but this was only on *Dolichos*, cowpea showing no response. "The position was unaffected at other sites", i.e. in five out of six instances lime-pelleting had no effect.

In order to look at this matter systematically it is necessary to be clear in our mind why we wish to lime-pellet. Is it the primary, secondary or tertiary function of the lime pellet, as outlined above, in which we are interested?

If it is the primary function, that of overcoming soil acidity, that interests us, then we are normally wasting our time lime-pelleting species like *Siratro* or *Centrosema* or *Lotononis* because they need no assistance in this matter. However, there may be exceptional circumstances in which a lime pellet is beneficial even for these species, as for instance if manganese toxicity is a factor. Thus Döbereiner and Aronovich (1965) found a beneficial effect of a lime pellet on nodulation of *Centrosema* in a manganese toxic sand in Brazil. Where we have tropical species such as *Leucaena* in which has occurred the same process of adaptation of the host to alkaline soils as has happened in clovers, lime-pelleting will very probably be beneficial but such species are few. In the case of borderline species like *Desmodium uncinatum* and *Glycine javanica* there may arise circumstances where the process will be beneficial but it is extremely doubtful if these will prove sufficiently numerous to justify the process as a routine measure. In the words of Parker and Oakley (1966) "the addition of a lime coat to the adhesive may further improve nodulation with certain legumes, and in certain circumstances, but we believe it should not be added as a routine procedure in the field until prior testing has shown it to be beneficial".

If it is the secondary function of the lime pellet, that of pre-inoculation, that interests us, then great caution is necessary in the case of tropical species. No-one has yet put forward evidence that the cowpea type of *Rhizobium* has any "staying power" inside a pellet comparable to that of clover or medic *Rhizobium*. It has to be remembered also that these species will normally be pelleted at a time when high temperature and high humidity combine to produce maximum death rate of *Rhizobium* (Vincent, 1958, 1965). But the chief difficulty lies in the fact that the slow-growing cowpea type of *Rhizobium* is an alkali-producer that is adapted to withstand acid and is consequently intolerant of alkalinity. The lime coating of the pellet is highly alkaline, and it is therefore a fundamentally unsound practice to subject this type of bacterium to a lime pellet. The bacteria will die faster under the lime coat than they will if simply applied to the seed with the adhesive. As an example of what may be expected, the results of an experiment with Greenleaf Desmodium are shown in Table 4. In the experiment seed was pelleted with a high count peat culture containing two strains, CB627 and CB1517, at a heavy rate estimated to give between 1,250 and 12,500 bacteria per seed. Survival of bacteria was measured by the percentage of plants nodulating in agar tubes at one day and again at one week. It can be seen that by one day the differential effect of the pelleting treatments had not yet been severe enough to influence results, but by one week the effect of the lime pellet was disastrous.

TABLE 4.
Nodulation resulting from different pellet treatment in *Desmodium intortum* (Greenleaf Desmodium)

Pellet material	% nodulation sown at 1 day	% nodulation sown at 1 week
None — 5% Cellofas sticker only	100	82
Lime	96	13
Gypsum	100	46
Nauru phosphate	100	57
Florida phosphate	96	61
Gafsa phosphate	100	52
Xmas phosphate	100	42

It should perhaps be mentioned that a result like this would not be obtained in a field experiment because this species normally nodulates quite well with native *Rhizobium* in the soil, and the failure of the *Rhizobium* in the lime pellet would be masked. The data of Shipton and Parker (1967) which appear to show unaffected survival of lupin and serradella bacteria under a lime pellet for 61 days are misleading since the stored pelleted seed received ten times the number of bacteria that were applied to the seed sown at one day.

TABLE 5.
A guide to pelleting material according to *Rhizobium* type

Species with acid-producing *Rhizobium* Use lime	Species with alkali-producing *Rhizobium* Use rock phosphate
Adesmia	*Acacia*
Anthyllis vulneraria	*Aeschynomene*
Astragalus	*Alysicarpus*
Cicer	*Arachis*
Coronilla varia	*Cajanus*
Dalea	*Calopogonium*
Hedysarum	*Canavalia*
Lathyrus	*Cassia*
Lens	*Centrosema*
Leucaena	*Clitoria*
Lotus corniculatus	*Crotalaria*
Lotus maroccanus	*Cyamopsis*
Medicago	*Desmodium*
Melilotus	*Dolichos*
Neptunia	*Glycine*
Onobrychis sativus	*Indigofera*
Phaseolus vulgaris	*Lespedeza*
Phaseolus coccineus	*Lotononis*
Pisum	*Lotus major*
Psoralea	*Lupinus*
Sesbania	*Ornithopus*
Trifolium	*Phaseolus* (all spp. except *P. vulgaris* and *P. coccineus*)
Trigonella	*Pueraria*
Vicia	*Rhynchosia*
	Stizolobium
	Stylosanthes
	Tephrosia
	Teramnus
	Vigna
	Zornia

On might conclude that the simplest thing to do is to use the adhesive only and in fact, if further research substantiates good survival of bacteria in lower concentrations of adhesive this may be a practical proposition. But at full strength there are too many problems introduced by the seed cementing together on drying. A compromise has been introduced by the use of rock phosphate dust instead of lime as a pellet coating for short-term pre-inoculation purposes. This material is readily available, it provides a source of calcium as a stimulant to nodulation, and it is more or less neutral in reaction, unless it has become contaminated with limestone during mining operations. A convenient guide to the use of lime or phosphate as a pellet according to type of *Rhizobium* possessed by the host legume is presented in Table 5. It is not complete since not all species have been categorized in this way at the present time, but it contains most species likely to be encountered by farmer, pastoralist or experimenter.

Finally, if it is the tertiary function of a lime pellet that is of interest, that is, if we wish to use it to allow mixing with superphosphate at planting, then a question of compromise is again involved. The data in Table 4 suggest that, provided a good peat culture is used, the loss of bacteria due to the unfavourable action of the lime will not be sufficiently fast to affect the issue if the seed is sown within a day of pelleting. There is, however, one complicating factor to be watched, which is seed quality. In a pelleting experiment similar to that in Table 4 the germination of lucerne and *Teramnus uncinatus* seed pelleted with 5% Cellofas A and lime was compared with that of untreated seed on 1% water agar plates. The germination figures were —

lucerne untreated	62%
lucerne pelleted	71%
Teramnus untreated	39%
Teramnus pelleted	20%

The *Teramnus* seed was an immature sample containing much pinched seed, and when the trial was repeated using a plump mature sample of seed, no inhibitory effect of lime was obtained.

However, the data of Murguia and Date (1965), who mixed rock phosphate pelleted seed with super for aerial sowing in Uruguay, indicate that for short term mixing the rock phosphate pellet will provide sufficient protection.

Summing up the situation, it might be said that lime-pelleting should be used intelligently and for a specific purpose, not as an act of faith. If used in the latter fashion, it can result on occasion in actual harm, with less nodulation and a lower germination in return for the cost and trouble of performing the operation.

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