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## NITROGEN FIXATION IN ACACIAS

Many a tree is found in the wood,  
And every tree for its use is good;  
Some for the strength of the gnarled root,  
Some for the sweetness of flower or fruit.

HENRY VAN DYKE, *Salute the Trees*

He that planteth a tree is the servant of God,  
He provideth a kindness for many generations,  
And faces that he hath not seen shall bless him.

HENRY VAN DYKE, *The Friendly Trees*



# Nitrogen Fixation in Acacias: an Untapped Resource for Sustainable Plantations, Farm Forestry and Land Reclamation

*John Brockwell, Suzette D. Searle, Alison C. Jeavons  
and Meigan Waayers*



Australian Centre for International Agricultural Research

2005

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## Foreword

Acacias possess many useful attributes — they are adapted to a wide range of warm-temperate and tropical environments including arid and saline sites, and infertile and acid soils. The species most used in forestry come from Australia or nearby countries, but others in cultivation are from India, Myanmar, Arabia, Africa, tropical America and Hawaii.

Although acacias have been an important element of agricultural systems in Africa for centuries, more extensive cultivation commenced after Australian species were introduced into India for fuelwood around 1850, and later to southern Africa for tanbark production. This latter role subsequently diffused, on a smaller scale, to other countries including Brazil and China.

Some hundred years later, the cultivation of acacias has again blossomed. Tropical species are attracting attention for their potential to provide wood for industrial and domestic purposes, and the use of a variety of species for land rehabilitation, especially in Australia, is expanding rapidly.

The ability of legumes to fix atmospheric nitrogen efficiently has been exploited with conspicuous success in agriculture — pastures based on subterranean clover are a notable Australian example. Such successes, and the prominence of acacias in many natural ecosystems, have fuelled hopes that effective nitrogen fixation by cultivated acacias would enhance the growth of both the acacia and associated crops.

Over the past two decades, Australian scientists and their counterparts in partner countries have pursued the domestication of acacias through a wide range of studies. Important outcomes include a greater understanding of both the acacias and their symbiotic micro-organisms, and the development of techniques for exploiting efficient symbioses. These developments are summarised in this review.

We compliment the authors of this publication. Their account is a valuable compilation of widely dispersed information, enhanced by their capacity to assess its merit and relevance. They conclude that improved nitrogen fixation is a potential bonus whenever acacia is cultivated, and that the magnitude of the bonus will depend on both effective inoculation and good silviculture.

ACIAR is pleased to continue its strong support for the domestication of these important trees through the publication of this review. The publication is also available on ACIAR's website at <[www.aciar.gov.au](http://www.aciar.gov.au)>.



Peter Core, Director, Australian Centre for International Agricultural Research



Alan Brown, Honorary Research Fellow, CSIRO Forestry and Forest Products





# Contents

<b>Foreword</b> .....	5
<b>Dedication</b> .....	9
<b>Preface</b> .....	11
<b>Acknowledgments</b> .....	13
<b>Abstract</b> .....	15
<b>1. Introduction</b> .....	17
<b>2. The plant</b> .....	18
Utilisation .....	19
Distribution .....	24
Plant taxonomy.....	24
Root and nodule morphology .....	28
<b>3. The bacteria</b> .....	29
Acacia rhizobia in nature.....	29
Taxonomy of acacia rhizobia .....	31
Diversity of acacia rhizobia .....	34
Comment on the taxonomy and diversity of acacia rhizobia.....	36
Root infection and nodule formation.....	36
Rhizobial products.....	37



<b>4. The symbiosis .....</b>	<b>39</b>
Measurement of nitrogen fixation .....	41
Consideration of procedures for measuring N fixation .....	43
Nitrogen fixation in glasshouse and nursery .....	44
Nitrogen fixation in the field .....	45
Environmental factors affecting nitrogen fixation in the field .....	49
Mycorrhizal factors affecting nitrogen fixation in the field .....	58
Rhizobial factors influencing nitrogen fixation in the field .....	60
<b>5. Enhancement of the symbiosis .....</b>	<b>69</b>
The need for inoculation.....	69
Responses to rhizobial inoculation of acacias in field and nursery .....	69
Inoculants .....	72
Breeding and selection for enhanced acacia symbiosis .....	77
<b>6. Exploitation of the symbiosis.....</b>	<b>78</b>
Natural exploitation .....	78
General exploitation .....	79
Intercropping .....	79
Land reclamation: principles and practice — the Australian experience.....	82
<b>7. General conclusions and prognosis.....</b>	<b>89</b>
The plant.....	89
The bacteria .....	90
The symbiosis.....	90
Exploitation .....	91
<b>References.....</b>	<b>94</b>
<b>Appendix .....</b>	<b>127</b>



## Dedication

This review is dedicated to the memory of Yvonne Barnet, Alan Gibson and Ben Bohlool. Each of these researchers was a pioneer in the field of nitrogen fixation in the genus *Acacia*, each was a distinguished scholar and teacher, and each died early and in tragic circumstances in the 1990s.

Dr Yvonne Barnet, working at the University of New South Wales, was among the very first to recognise the extremes of morphological diversity within acacia root-nodule bacteria. Her observation was one of the catalysts that eventually led to extensive taxonomic reclassification of the Rhizobiaceae. Early in his career, Dr Alan Gibson, CSIRO Plant Industry, Canberra, conducted ground-breaking research on the physiological effects of environmental variables on the efficiency of legume nitrogen fixation. Later in life, he developed an interest in host/plant specificity in the *Acacia*/rhizobia symbiosis and,

at the time of his death, was making meaningful progress towards unravelling its complexities. Professor Ben Bohlool was Director of the University of Hawaii's NifTAL Project (Nitrogen Fixation by Tropical Agricultural Legumes), located at Paia, Maui, Hawaii, a centre famous for its international training courses and workshops. Ben Bohlool's boundless enthusiasm instilled a lifelong interest in nitrogen fixation in literally hundreds of people. Many of them have added to current knowledge of the acacia symbiosis.

During their lifetimes, Yvonne Barnet, Alan Gibson and Ben Bohlool each made substantial contributions to research on legume nitrogen fixation and the rhizobial symbiosis of the genus *Acacia*. Without them, our understanding of the field would be diminished. We deeply regret their passing.



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## Preface

It is a curiosity that, in Australia with its great wealth of native plants, so little science apart from taxonomy has been devoted to this unique flora. This observation applies particularly to studies of the nitrogen-fixing symbiosis between Australian native legumes and their root-nodule bacteria. This is all the more strange when it is considered that, as early as the late 1950s, Rob Lange, as a postgraduate student in the late Lex Parker's laboratory at the University of Western Australia in Perth, began publishing fascinating, thought-provoking papers on the diversity of legumes and root-nodule bacteria indigenous to the soils of the south-west of Western Australia. Of course, the field has not been completely neglected. Ann Lawrie in Melbourne and the late Yvonne Barnet in Sydney maintained an ongoing interest and the occasional paper from other Australian sources has appeared in the literature from time to time. In addition, the Australian Centre for International Agricultural Research (ACIAR) has sponsored several international conferences to deal with manifold aspects of the utilisation of *Acacia* species around the world; from time to time, papers about the acacia symbiosis have appeared in the proceedings.

This monograph arose from an ACIAR project, 'Australian acacias for sustainable development in China, Vietnam and Australia — Subproject B.

Effectiveness and persistence of *Acacia* rhizobia', when one of us (John Brockwell) was thrown in at the deep end of the field following the untimely death of Alan Gibson, a principal investigator for the subproject. A first step in the learning process was a review of the literature. This turned out to be more substantial than we had imagined and highly dispersed in terms of the species investigated, the aspects of the symbiotic relationship studied and the places of publication of results. There appeared to be three main reasons for this: first, the genus is so large — some 1350 species; second, it is more difficult to experiment with trees and shrubs than it is with herbs; and third, it has been unfashionable until recent times to work with nitrogen-fixing trees. It then occurred to us that it would be helpful to our incomplete but growing understanding of the field if we were to bring the literature together in the form of a review. This monograph is the result. We hope it will be useful to wider audiences. Our different areas of experience have been conducive to a fruitful, complementary collaboration. John Brockwell has worked for many years on a number of aspects of symbiotic nitrogen fixation in crop and forage legumes in agricultural settings and the utilisation of fixed nitrogen in phase-farming systems. Suzette Searle's extensive dealings with Australian acacias have been directed at distribution, intraspecific variation,

climatic, topographic and edaphic adaptation, and utilisation for farm and plantation forestry in both Australia and Asia. Alison Jeavons and Meigan Waayers are working at the forefront of sustainable land reclamation and are using acacias and other Australian native trees and shrubs, and various establishment techniques, for both large- and small-scale revegetation of damaged lands.

There is, of course, a plethora of literature dealing with legume nitrogen fixation. Nonetheless, we feel that the appearance of this monograph is timely. At the time of writing the preface (mid-2004), world crude oil prices have risen to record levels, reminding us once again, if that were ever necessary, that fossil fuels and their nitrogenous fertiliser by-products are a finite resource. Anything that can be done to utilise nitrogen biologically fixed from the atmosphere to replace or supplement the use of fertiliser nitrogen must be of ultimate benefit to the human race and the environment. Estimates of the amounts of legume nitrogen fixed worldwide go as high as 100 million tonnes annually. Acacias represent 6–7% of the more than 20,000 known species of legumes and must make a very substantial contribution to the total amount of nitrogen that

is fixed on Earth. Yet, the potential for exploiting acacia nitrogen fixation has been almost completely overlooked. We hope that this monograph will stimulate interest in tapping into this neglected resource.

At times, we have been emphatic and contentious. That has been deliberate and we don't apologise. It has been our aim to stimulate discussion and ideas. If we can encourage even a small number of people to interest themselves in, and perhaps study, the hugely diverse and fascinating genus that is *Acacia*, its equally diverse root-nodule bacteria and their capacity together to fix nitrogen from the infinite resource of the Earth's atmosphere, then we will have achieved our objective. As for ourselves, we enjoyed the experience of compiling the monograph. Our review of the literature has taken us into quite new terrain. We cannot help but admire those people who have made observations and conducted field experiments, some quite sophisticated, in difficult circumstances and in remote and sometimes dangerous places and, in so doing, started to unravel the complexities of the symbiosis between *Acacia* and its root-nodule bacteria.



# Acknowledgments

Many people helped us, in small ways and large, in compiling the monograph; we mention here only a few of them. We are greatly indebted to Mike Trinick, formerly CSIRO Plant Industry, Canberra, for allowing us to make use of his database of historical literature references to acacia symbioses, and to Michelle Hearn, CSIRO Black Mountain Library, Canberra, for her patience in unearthing obscure references. Partap Khanna, CSIRO Forestry and Forest Products, Canberra, kindly allowed us to use some of his unpublished data on relative growth rates of *Acacia* and *Eucalyptus* growing in mixed stands that showed how the eucalypts responded to the presence of acacias that were actively fixing nitrogen. In this same context, we are pleased to acknowledge the editor of *The Canberra Times*, a newspaper published daily in Canberra, ACT, Australia, for permission to reproduce a cartoon relating to the benefits of growing species of *Acacia* and *Eucalyptus* in mixtures. We are indebted to Janet Sprent, University of Dundee, UK, Ken Giller, University of Wageningen, The Netherlands, and Margaret Thornton, CSIRO Black Mountain Library, Canberra, for assisting us in our efforts to keep abreast of the recent rapid developments in the taxonomy of the legume root-nodule bacteria. We are grateful also to Paul Singleton and the NifTAL Center and MIRCEN, Paia, Hawaii, USA, for supplying us with details of NifTAL's 'micro-

production unit' for inoculant preparation, to Gary Bullard, formerly Bio-Care Technology Pty Ltd, Somersby, New South Wales, for providing a manufacturer's perspective of essential characteristics of good legume-inoculant strains, and to Jackie Nobbs, SARDI, Adelaide, for providing the authority for the nematode name, *Meloidogyne javanica*. We thank Sandra McIntosh and Siobhan Duffy, CSIRO Plant Industry Visual Resources Unit, Canberra, for preparing the illustrations. In particular, we are most grateful to Mark Peoples, CSIRO Plant Industry, Canberra, for allowing us to make use of some of his unpublished results, to Janet Sprent, University of Dundee, UK, for very helpful discussions and her encouragement, and to Alan Brown, CSIRO Forestry and Forest Products, Canberra, and Jeremy Burdon, CSIRO Plant Industry, Canberra, for their critical appraisals of the manuscript.

The monograph contains a number of photographs. Less than half of them are our own. We are especially indebted to those people who allowed us access to their files of photographs and generously gave us permission to reproduce them. Sources are acknowledged with the photographs.

In compiling this review, it was never our intention to have an exhaustive list of citations to the

## NITROGEN FIXATION IN ACACIAS

nitrogen-fixing symbiosis in the genus *Acacia*. It is certain, however, that we must have overlooked some works of substance that should have been included. We apologise to the authors concerned. In

addition, we take full responsibility for other errors and omissions and will be grateful to whomsoever draws them to our attention.



Alison Jeavons

*Acacia paradoxa*



## Abstract

The legume genus *Acacia* has some 1350 species and is distributed throughout the world, particularly in Africa, Asia and Australia. The genus is exploited in natural habitats and plantations for many purposes. It forms a symbiotic association with strains of at least six genera of root-nodule bacteria (rhizobia) that are also widely distributed. Many of the associations fix nitrogen from the atmosphere, but there is great variation in nitrogen-fixing specificity in both hosts and bacteria — some acacias fix nitrogen with only a small number of rhizobial strains, others are more promiscuous.

Records indicate that acacias fix less nitrogen than other leguminous trees. However, this impression appears to be an artefact of the ecosystems where the measurements were made. Most assessments of acacia nitrogen fixation have been undertaken in forests or woodlands where nitrate in the soils often inhibits nitrogen fixation, whereas the nitrogen fixed by other tree legumes has usually been measured in anthropogenic ecosystems such as plantations, hedgerows and coppices, where soil nitrate is less inhibitory. We conclude that acacias have the capacity to fix useful quantities of nitrogen but that, unlike the plant itself, its symbiosis is under-utilised.

Factors that might limit nitrogen fixation are considered, with the conclusion that, as with other legumes, nitrogen-fixing ability is best expressed in the absence of limiting factors, especially deficiencies of nutrients and soil moisture. There is usually a diversity of strains of rhizobia in soils where acacias grow naturally. Many of these strains do not nodulate *Acacia* spp. at all and many others that do form nodules have little or no capacity to fix nitrogen. However, it appears that, within the total population of naturally occurring rhizobia, there are invariably present at least some strains that are capable of fixing significant amounts of nitrogen in association with acacias. There is no convincing evidence that, in natural environments, non-infective, ineffective or poorly effective rhizobia themselves are ever a constraint on acacia nitrogen fixation.

We conclude that little can be done to enhance acacia nitrogen fixation in forests or established plantations except, where economically feasible, to correct nutrient imbalances and to control pests. On the other hand, there appears to be great and inexpensive scope to use inoculation with effective strains of rhizobia to improve the vigour and nitrogen fixation of seedlings grown in plant nurseries as tube stock destined for outplanting into the field. Where outplantings are made, inoculated,



well-nodulated seedlings survive better and grow faster than their uninoculated counterparts. Inoculation with rhizobial strains cultured in a peat carrier, using a procedure termed soil enrichment, is postulated as an efficient means of producing vigorous, well-nodulated, nitrogen-fixing acacia seedlings in nurseries. Implications for commercial manufacture of acacia inoculants are discussed.

Three factors are especially relevant to the timeliness and significance of this review: (i) the already substantial, and expanding, scale of acacia plantings in plantations and on farms, (ii) the potential of diverse *Acacia* species for the reclamation of degraded landscapes, and (iii) the expanded pool of research results relating to acacias generally that has accumulated over the past 15 years.

In conclusion, there are compelling arguments that acacia nitrogen fixation can be far better exploited than it has been in the past. This will involve effective rhizobial inoculation of seedling stock in nurseries and development of methods of inoculation of acacia seed intended for surface seeding. There seems no doubt that, properly exploited, the symbiosis has the capacity to contribute to the productivity of acacia and companion species in plantations, to the rehabilitation of eroded and salinised lands, and to the augmentation of reserves of nitrogen in the soil. Even in circumstances where inoculation is not practicable, the cultivation of acacias has the potential to enhance soil fertility and soil structure.



Alison Jeavons

*Acacia podalyriifolia*

# 1. Introduction

The world uses huge quantities of synthetic nitrogenous fertiliser for growing plants. This dependence creates certain dangers for the global economy and especially for the environment (Smil 1997). Anything that can be done to utilise nitrogen (N) fixed naturally from the atmosphere — where it occurs as molecular nitrogen ( $N_2$ ) — as a substitute for fertiliser N, will benefit all people.

*Acacia* species are legumes and, in symbiotic association with root-nodule bacteria, are partners in fixation of atmospheric N (N fixation). Estimation of the total quantity of legume N fixed worldwide is an exercise in informed guesswork, but the amount is of the order of 70–100 million tonnes annually. Since the known number of *Acacia* species represents some 6–7% of the 20,000 species of legumes, acacias must make a substantial contribution to the total quantity of N fixed in terrestrial natural systems.

Duke (1981) lists six species of *Acacia* in his 'Handbook of legumes of world economic importance' and many others are utilised in a multiplicity of ways by humans and all types of animals. However, little effort has been made to exploit the N-fixing characteristic of the genus. The purpose of this review is to ask why, and to consider what means might be employed to increase N

fixation by acacias to benefit their productivity and the sustainability of the natural and anthropogenic ecosystems in which they grow.

We consider first some literature relating independently to the plant and its root-nodule bacteria, then deal with the symbiotic association between them. We observe that, in many respects, the processes of N fixation in acacias are similar to those that apply to legumes generally. Therefore, when we could find no literature relevant to acacias, we have drawn upon information for other legumes. Finally, we speculate on how the acacia symbiosis might be exploited.



*Acacia aneura* (mulga) is widely distributed in the Australian arid zone. Its foliage is browsed by ruminants, especially in times of drought, and its wood is valued for turning and carving.



## 2. The plant

Legumes first appeared on Earth some 70 million years ago (Polhill et al. 1981). The legumes comprise three families, viz. Fabaceae, Mimosaceae and Caesalpinioideae, though some authorities, e.g. Lewis et al. (2001), prefer to regard the legumes as a single family (the Leguminosae or Fabaceae) with three subfamilies — Papilionoideae, Mimosoideae and Caesalpinioideae. Within the Mimosaceae, *Acacia* is much the largest genus (and apparently still growing as new species are recognised) — estimated at some 1200–1300 species by Chappill and Maslin (1995) and at 1350 species less than nine years later (Turnbull 2004). Nearly 1000 of these occur naturally and only in Australia (Maslin and Hnatiuk 1987; Maslin and McDonald 1996). ‘The Flora of Australia’ lists 955 species of *Acacia* (Orchard and Wilson 2001a,b). *Acacia* is also widely distributed in Africa (about 144 species), Asia (about 89 species) and the Americas (about 185 species) (Maslin and Stirton 1997; Orchard and Maslin 2003; Turnbull 2004), but acacias are rare in Europe. The genus is not indigenous to New Zealand and smaller islands of the Pacific (Greenwood 1978), but is often an introduction. Acacias range from herbs (rare) to enormous trees — see e.g. Menninger (1962), but most are shrubs and small trees. Their habitats range from arid areas of low or seasonal rainfall to moist forests and river banks (Allen and Allen 1981). Species are found on all soil types.

The *Acacia* genus includes some of the world’s most beautiful plants. In flower, *Acacia baileyana*<sup>1</sup>, *A. podalyriifolia* and *A. pycnantha* are fully clad in a raiment of fluffy, golden yellow balls that persist for several weeks. Indeed, *A. pycnantha* is Australia’s floral emblem and Wattle Day is celebrated in some States as the first day of spring. The springtime fragrance of *A. mearnsii* is a sensuous feature of the bushland of eastern Australia. There is a grand stateliness of form of *A. dealbata* and *A. melanoxylon* in mature forests. The graceful foliage of *A. excelsa* and the pensile symmetry of *A. pendula* create park-like settings in the pastoral lands of western New South Wales. For a traveller in arid country to come upon *A. cambagei* in full flower is an experience to stir the soul. Low-growing acacias act as shelter, sanctuaries, and feeding and breeding grounds for small, native mammals and birds. Seedlings make rapid early growth. It is small wonder, then, that acacias are sought after as shrubs and trees for home gardens, parks and roadside verges, and to provide visual screens, shade, shelter belts and wildlife habitats.

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<sup>1</sup> Throughout the manuscript, we usually refer to acacias and other plant species by their botanical names. Common names and authorities for botanical names are given in the appendix.

## UTILISATION

Aesthetics aside, the uses to which *Acacia* species and acacia products are put are manifold. We will dwell only briefly and selectively on these. The subject has been well reviewed for N-fixing trees in general by Dommergues et al. (1999) and for acacias in particular by Thomson et al. (1994), Searle (1996), Turnbull et al. (1998a), McDonald et al. (2001) and Maslin and McDonald (2004). The genetic resources of useful and potentially useful acacias have been recorded by Pinyopusarerk (1993). Some examples of the wide range of uses of the genus are given in Table 1.



Alison Jeavons

*Acacia pycnantha* (golden wattle) is Australia's floral emblem.

**Table 1.** Utilisation of (selected) *Acacia* species

Species	Uses
<i>A. acuminata</i>	Charcoal, wood turning
<i>A. albid</i> <sup>a</sup>	Fodder <sup>b</sup> , soil enrichment
<i>A. aneura</i>	Fodder, posts, turning, bush food <sup>c</sup>
<i>A. auriculiformis</i>	Environmental rehabilitation, soil stabilisation, fuel wood, posts, pulpwood
<i>A. baileyana</i>	Cut flowers/foilage, pollen, gum
<i>A. berlandieri</i>	Gum
<i>A. cambagei</i>	Posts, turning, bush food
<i>A. catechu</i>	Fuel wood
<i>A. crassicarpa</i>	Tolerance of high water tables
<i>A. cyclops</i>	Salinity tolerance
<i>A. dealbata</i>	Pulpwood, gum, cut flowers/foilage, oils, pollen
<i>A. decurrens</i>	Pulpwood, timber, fuel wood
<i>A. elata</i>	Pulpwood
<i>A. excelsa</i>	Fodder
<i>A. gerrardii</i>	Fodder
<i>A. harpophylla</i>	Posts, fuel wood, charcoal
<i>A. hebeclada</i>	gum (acidic)
<i>A. homalophylla</i>	Turning

**Table 1.** (cont'd) Utilisation of (selected) *Acacia* species

Species	Uses
<i>A. imbricata</i>	Cut flowers/foilage
<i>A. implexa</i>	Fuel wood, turning, pollen
<i>A. irrorata</i>	Fuel wood, tannin
<i>A. kempeana</i>	Fodder
<i>A. leucophylla</i>	Non-industrial wood <sup>d</sup>
<i>A. mangium</i>	Timber, pulpwood
<i>A. mearnsii</i>	Tannin, oyster poles, mine timber, pollen, fuel wood, charcoal, craft wood, pulpwood, mushroom medium, adhesives, cellulose for rayon, particle board
<i>A. melanoxylon</i>	Joinery, turning, pulpwood
<i>A. mellifera</i>	Fodder, honey
<i>A. nilotica</i>	Non-industrial wood
<i>A. notabilis</i>	Bush food
<i>A. papyrocarpa</i>	Bush food, turning
<i>A. parramattensis</i>	Pulpwood, tannin
<i>A. pendula</i>	Fodder, turning, fuel wood
<i>A. pycnantha</i>	Tannin, gum, bush food
<i>A. retinodes</i>	Salinity tolerance, cut flowers/foilage, bush food
<i>A. salicina</i>	Salinity tolerance, soil stabilisation, joinery
<i>A. saligna</i>	Salinity tolerance, soil stabilisation, tannin, fodder, bush food, (acidic) gum, fuel wood
<i>A. senegal</i>	Gum <sup>e</sup>
<i>A. seyal</i>	Non-industrial wood
<i>A. silvestris</i>	Pulpwood, joinery, fuel wood, posts, pollen, bush food, cut flowers/foilage, tool handles
<i>A. stenophylla</i>	salinity tolerance, fodder, posts, fuel wood
<i>A. tortilis</i>	Fodder, posts, fuel wood, soil stabilisation, charcoal
<i>A. trachyphloia</i>	Pulpwood
<i>A. verniciflua</i>	Cut flowers/foilage
<i>A. victoriae</i>	Fodder, bush food, pollen

<sup>a</sup> More properly *Faidherbia albida*.

<sup>b</sup> Fodder refers to foliage or fruits grazed or browsed by domestic animals.

<sup>c</sup> Bush food is foodstuff utilised for human consumption through the Australian bush food industry, mainly as flavourings.

<sup>d</sup> The term 'non-industrial wood' implies a number of uses including joinery, turning, carving, fencing, charcoal making and fuel wood; it excludes wood suitable for milling as construction timber.

<sup>e</sup> Once widely used, as gum arabic, as adhesive for coating legume seed as an aid to inoculation (Brockwell 1962).

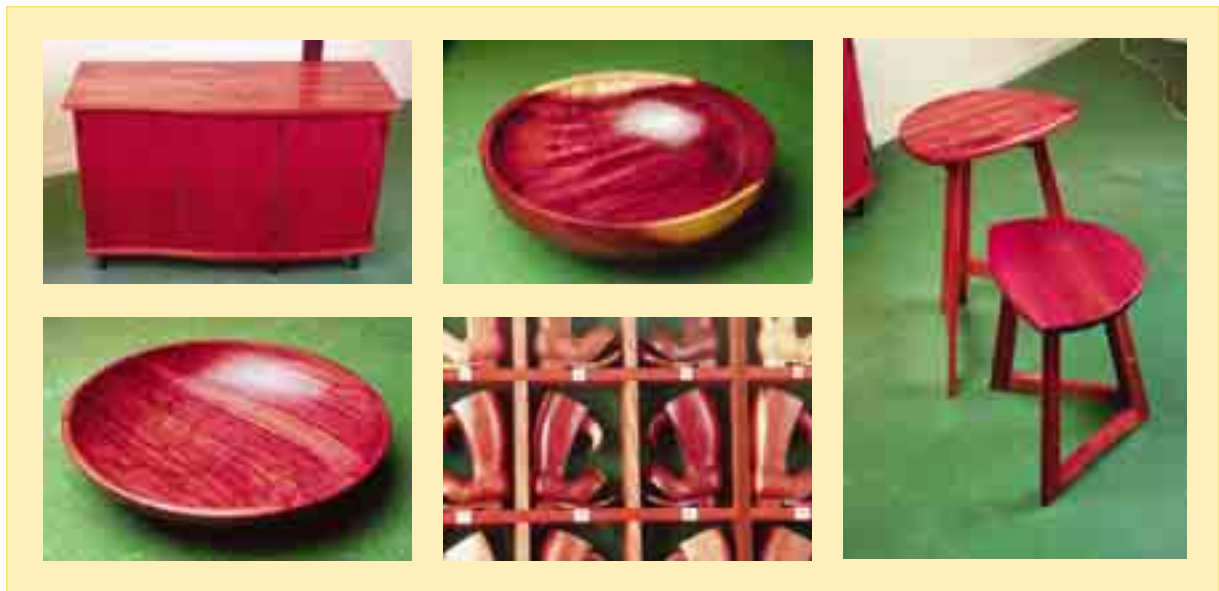
### Wood products

The use of acacia wood dates back to ancient times. Historians are in general agreement that the religious icons, the 'Ark of the Covenant' and the 'Altar and Table of the Tabernacle', were constructed from timber cut from *A. seyal* and/or *A. tortilis* (Moldenke and Moldenke 1952).

Relatively few species of *Acacia* grow large enough for construction timber or furniture making. However, one of these is *A. melanoxylon* which has a wide latitudinal range in Australia (Searle 1996) and which, in optimum climates, may grow up to 35 m in height and 1.5 m in diameter (Boland et al. 1984). The common name of the species, (Tasmanian)

blackwood, barely does justice to the beauty of its heartwood which is prized for furniture making, joinery and turning.

*Acacia mangium* and *A. auriculiformis* have been widely planted in the tropics of Asia, the former in plantations for wood-pulp production, the latter mainly by smallholders for non-industrial wood (Turnbull et al. 1998b). *Acacia auriculiformis* is adapted to infertile soils, including the large areas of degraded (*Imperata*) grasslands in Southeast Asia. Poor stem form has restricted its use, but there is potential for the exploitation of provenances with straight stems (Venkateswarlu et al. 1994) and of its hybrids with *A. mangium* (Kha 1996).



David McClenaghan

Products from acacia timbers, courtesy of the Bungendore Wood Works Gallery, Bungendore, New South Wales

Many acacias make excellent fuelwood and charcoal (Searle 1995). Fence posts are cut from a number of species (Searle 1996), some of which, e.g. *A. cambagei*, are resistant to termite attack. Poles of *A. mearnsii*, cut with the bark intact, are used in oyster farming; the high tannin content of the bark apparently delays degeneration of the poles induced by marine borers (Searle 1996). Other acacia wood products include chips for wood-pulp manufacture of paper, rayon and particle board (Sherry 1971; Hillis 1996; Mitchell 1998) and for sawdust as a medium for cultivation of edible fungi (Lin 1991).

### Non-wood products

Gums exuded from higher plants, including acacias, are complex carbohydrates (Anderson et al. 1971, 1984) that are used in food processing and medicines. Gum arabic, perhaps the most commercially important of the natural gums, is a product of the tree *A. senegal*, growing mainly in Africa (National Academy of Sciences 1979).

The tannins contained in certain wattle barks are used in water- and weather-proofing processes and in the leather industry (Sherry 1971; Yazaki and Collins 1997). The bark of *A. mearnsii* is particularly rich in tannins. In Australia, millions of trees were stripped of their bark to supply tanneries and export markets between the 1880s and the 1960s (Searle 1991). In South Africa, substantial plantations of *A. mearnsii* grown specifically for tannin extraction make that country the world's major exporter of powdered vegetable tannin (Sherry 1971).



Courtesy of CSIRO Forestry and Forest Products

A plantation of *Acacia mearnsii* (black wattle) near Eldoret Eatel, Kenya. The bark of *A. mearnsii* is a prolific source of industrial tannin.

Humans and animals eat acacias. A conference in the early 1990s examined the potential of acacias as sources of human foodstuff (House and Harwood 1992). Edible acacia seeds have since been documented (Maslin et al. 1998). Australia's Indigenous people used at least 44 species of *Acacia* for food (Thomson 1992). This knowledge is now being utilised in West Africa (Harwood 1999; Midgley and Turnbull 2001). To explore the likelihood that seeds of Australian acacias may prove a useful dietary supplement for human consumption in the Sahel region, the Australian Government in 1997 funded two Aboriginal women from central Australia to visit an indigenous community in Niger to exchange information (Australian Department of Primary Industries and Energy 1997). The outcome was productive (cf. Harwood et al. 1999). Leaves of *A. pennata* subsp. *insuavis* are used for culinary and medicinal purposes in Thailand and India (Bhumibhamon 2002). Wattle seed is now

## NITROGEN FIXATION IN ACACIAS

one of the boutique Australian bush foods used as a flavouring agent (ANBIC 1996). Grazing animals browse several species of *Acacia* especially *A. saligna*. *Acacia aneura* foliage is used for feeding sheep and cattle in times of drought (Norton 1994). *Acacia* pollen is a source of protein for honey bees (Boland 1987). In Australia, several species are cultivated for cut flowers and foliage for both domestic and export markets (Sedgley and Parletta 1993). Fragrant



John Brockwell

*Acacia cambagei* (gidgee) is a common tree in semi-arid and arid Australia and may grow to 15 metres. Its wood is hard and resistant to termites and is often used for fence posts.

oils extracted from the flowers of *A. dealbata* and *A. farnesiana* are valued as blenders and fixatives in perfume manufacture (Boland 1987).



John Brockwell

*Acacia peuce* (waddy wood) is a rare and endangered species (Leigh et al. 1981). It occurs in small, disjunct populations at three general locations, all in inland Australia in very arid environments. This specimen was photographed in 1999 at a reserve dedicated to *A. peuce* near Birdsville, Queensland. The mean annual rainfall there is 140 mm.



Many acacias are used for environmental rehabilitation (Doran and Turnbull 1997). For example, they are grown on overburden from mining activities (Langkamp et al. 1979) and are used to lower watertables beneath saline soils, including those subject to waterlogging (Biddiscombe et al. 1985; Ansari et al. 1998; Marcar et al. 1998), in soil stabilisation (Searle 1996; Harwood et al. 1998), and to increase productivity of degraded grassland (Turnbull et al. 1998b). Many Australian acacias are fire tolerant (Dart and Brown 2001), which is a particular advantage for land rehabilitation in wildfire-prone environments.

## DISTRIBUTION

In addition to the natural global distribution of the genus, acacias are widely grown in plantations. Extensive plantings have been established in China, India, Indonesia, Malaysia, the Philippines, Thailand, Vietnam (Turnbull et al. 1998b) and South Africa (Sherry 1971). The largest plantations are in India where a total area of three million hectares (Pandey 1995) has been planted, mainly with the spiny species *A. catechu*, *A. leucophloea*, *A. nilotica* and *A. tortilis* (Hocking 1993). There are approximately two million hectares planted worldwide (Table 2) with non-spiny Australian acacias, mostly *A. mangium*, *A. mearnsii* and *A. saligna* (Maslin and McDonald 1996; Midgley and Turnbull 2001). In the past, Australia had few acacia plantations because trees were so readily available in the wild (Searle 1995). That situation now appears to be changing (Mitchell 1998; Neilson et al. 1998; Byrne et al. 2001).

**Table 2.** Plantings of some Australian species of *Acacia* in other parts of the world

Species	Approximate total area (ha)	Whereabouts of major plantings
<i>A. crassicarpa</i>	50,000	Sumatra
<i>A. mangium</i>	800,000	Indonesia, Malaysia
<i>A. mearnsii</i>	450,000	South Africa
<i>A. saligna</i>	500,000	North Africa, West Asia, Chile

Source: after Midgley and Turnbull (2001).

In field trials, acacias often grow more quickly than other N-fixing trees. For example, in the first 12 months after outplanting at three locations in Rwanda, the growth of *A. mearnsii* was superior to that of eight other species (Uwamariya 2000). Natural hybridisation sometimes occurs between *Acacia* species, accompanied by enhancement of plant vigour. Kha (2000) reported from Vietnam that the stem volumes of *A. mangium*/*A. auriculiformis* hybrids were about three times as great as those of the parents. A factor in the improved growth may have been that the root nodules of the hybrids were 2–4 times greater in number and weight, and perhaps in N-fixing capacity, than the nodules of the parental species.

## PLANT TAXONOMY

The type species of the genus *Acacia* is *A. arabica* (now *A. nilotica* subsp. *nilotica*) which is native to the northern half of the African continent. The botanical name *Acacia* is derived from the Greek

'akis' meaning point or barb, a reference to the spines. The derivation is apt for African species which are mainly spiny but not for Australian species which, as a rule, have no spines.

Pedley (1986) suggests a plausible scenario for the evolution of *Acacia* and its subgenera and sections. Amongst the legumes, the cosmopolitan, polyphyletic genus *Acacia* (some 1350 species) is second only to *Astragalus* in numerical size. The very size of the genus is further complicated by a substantial degree of outcrossing, e.g. *A. auriculiformis* (Khasa et al. 1993), *A. crassicarpa* (Moran et al. 1989), *A. decurrens* (Philp and Sherry 1946), *A. mearnsii* (Moffett 1956) and *A. melanoxylon* (Muona et al. 1991). Moreover, in some species, there is a wide range of intra-specific differentiation and diversity, both genetic and morphological, e.g. *A. acuminata* (Byrne et al. 2001), *A. aneura* (Andrew et al. 2001) and *A. tumida* (McDonald et al. 2001). The differentiation within *A. aneura* is so marked that the species itself is considered a 'complex' (Andrew et al. 2001). Taxonomic revision of such a genus is probably inevitable. Indeed, a major revision was proposed nearly 20 years ago (Pedley 1986) but not widely accepted, perhaps because it would be 'so disruptive' (Maslin 1995). Nonetheless, many of Pedley's (1978) earlier and less-controversial proposals have been adopted for the treatment of acacias in the 'Flora of Australia'. This is not to say that revision of some sections and individual species is not proceeding. For instance, McDonald and Maslin (1998) summarised a proposal for a taxonomic revision of *A. aulacocarpa* and its close relatives. The outcomes of these

deliberations have been comprehensively dealt with by Orchard and Wilson (2001a,b) in the 'Flora of Australia, Mimosaceae, *Acacia*'.

Also, the tree once regarded as *A. albida*<sup>2</sup> is now properly known as a *Faidherbia* (*F. albida*), a monospecific genus (Vassal 1981) distributed throughout Africa and adjoining regions of West Asia.

A simplistic version of the classification of legumes, with particular reference to Australian acacias, is illustrated in Figure 1. Of the nearly 1000 species of *Acacia* recognised as Australian, the majority are contained in the subgenus *Phyllodineae*. The remainder are accommodated in subgenera *Acacia* (180–190 species) and *Aculeiferum* (120–130 species) (Maslin 2001).

Although the rhizobial symbiosis is not a classical systematic criterion in legume taxonomy, it is now widely recognised that it does have substantial relevance (e.g. Sprent 2001b). Norris (1959) held the view that the symbiotic characters, nodulation and N fixation, expressed during the interaction between African species of *Trifolium* and strains of *Rhizobium leguminosarum* bv. *trifolii* (t'Mannetje 1967), were useful in the taxonomy of both plants and bacteria. He called his concept 'symbiotaxonomy'. The concept has already found application in the classification of biovars of *R. leguminosarum* (Kreig and Holt 1984).

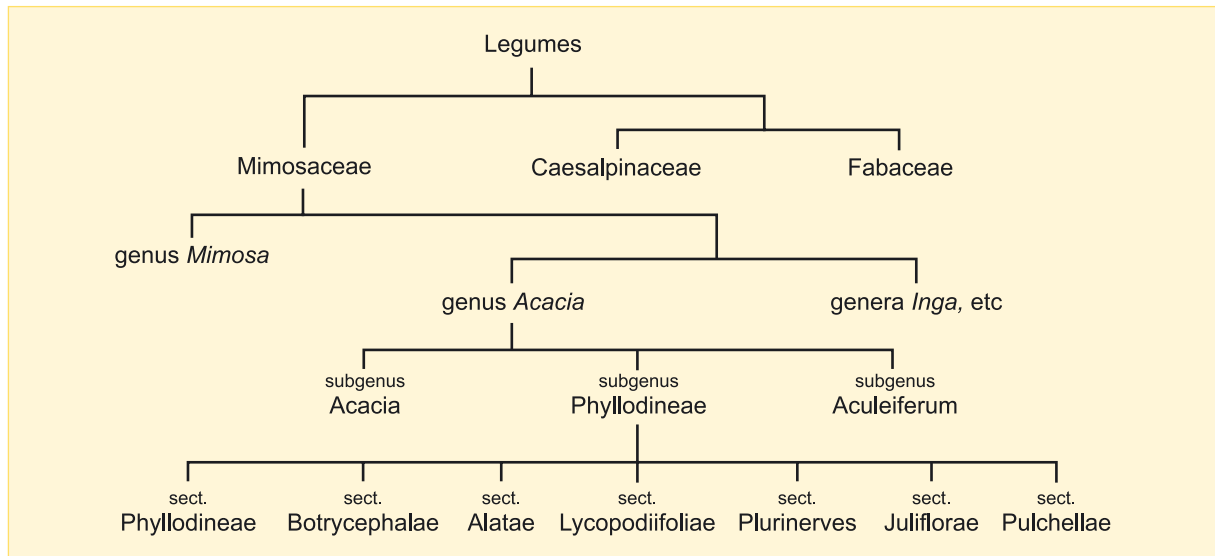
<sup>2</sup> As a matter of convenience throughout this review, we use the name *A. albida* instead of *F. albida*, although we recognise that it is obsolete.



A spectacular example of the association between symbiotaxonomy and legume taxonomy was the reclassification of the complex genus formerly recognised as *Phaseolus* (Fabaceae). Legume bacteriologists had long been aware of paradoxes within the complex, viz. different species had fast- and slow-growing root-nodule bacteria and there were marked host/bacteria specificities in nodulation and N fixation. All this was resolved by Verdcourt (1970a,b). He defined several genera within the *Phaseolus* complex including *Phaseolus*, nodulated by fast-growing species of *Rhizobium* (now known to include *Rhizobium leguminosarum* bv. *phaseoli*, *R. etli* bv. *phaseoli*, *R. gallicum* bv. *gallicum*, *R. gallicum* bv. *phaseoli*, *R. giardinii* bv. *giardinii*, *R. giardinii* bv. *phaseoli*,

*R. tropici*) and *Macroptilium*, *Macrotyloma* and *Vigna*, nodulated by slow-growing *Bradyrhizobium* species. Symbiotaxonomy apparently also has a potential role in the systematics of *Lotus* (also Fabaceae) and symbiotically related genera (Brockwell et al. 1994).

There appears to be some relatedness between taxonomic classification of the Caesalpiniaceae and symbiotic characteristics. Most species within the Caesalpiniaceae do not nodulate (Allen and Allen 1981). However, there are exceptions within the genus *Cassia* which contains both nodulating and non-nodulating species. When the leguminous tribe Cassieae subtribe Cassiinae underwent taxonomic revision (Irwin and Barnaby 1982; see also Randall and Barlow 1998a,b), three separate genera, viz.



**Figure 1.** A simplistic illustration of taxonomic arrangements within the genus *Acacia*, with special reference to Australian species — after Tame (1992), derived in part from Pedley (1978). This is a practical classification but it does not necessarily reflect the phylogeny of the genus *Acacia*.

*Cassia*, *Senna* and *Chamaecrista*, were recognised, and some species, formerly *Cassia*, were allocated to *Senna*. Two of those were the non-nodulating species, now *Senna siamea* and *S. spectabilis*. It is consistent with principles of symbiotaxonomy that no species within the genus *Senna* have been found to nodulate (Allen and Allen 1933; Giller and Wilson 1991), although the search has not been exhaustive. On the other hand, it is inconsistent with those principles that the genus *Cassia* appears still to contain both nodulating and non-nodulating species.

Until recently, symbiotaxonomic criteria had not been considered within the Mimosaceae. It now appears from the work of Harrier et al. (1997) that a taxonomically distinct group of *Acacia* species native to Africa is unable to form nodules. There are also indications (de Faria and de Lima 1998) that another group of acacias that occurs naturally in Central and South America is similarly unable to nodulate. These particular species are members of the subgenus *Aculeiferum* ser. *Americanae*. Paradoxically, however, other species within the *Americanae* have been reported as bearing nodules. It will be of interest to learn whether taxonomic botanists may, sometime in the future, uncover systematic criteria that separate these non-nodulating groups from nodulating *Acacia* species at the generic level. Further, more specific information about non-nodulation in acacias is presented later, in the section entitled ‘The bacteria — acacia rhizobia in nature’.

It seems inevitable that the large, complex genus known as *Acacia* will eventually be revised into

several smaller genera. The arguments of Pedley (1986) and Chappill and Maslin (1995), and the DNA-based evidence of Miller and Bayer (2001), for doing so are compelling. (We have already noted the reclassification of *Acacia albida* to *Faidherbia albida*.) In Australia, debate about how the revision should be done has led to a polarisation of opinion. On the one hand, Pedley (1986) believes *inter alia* (i) that the existing type species, *A. nilotica*, should be conserved, with most of the African species retaining the generic name *Acacia*, and (ii) that the largest existing subgenus *Phyllodineae*, which contains most of the 1000 Australian species, should become genus *Racosperma*. Orchard and Maslin (2003), on the other hand, contend that means should be found to limit the extent of change that would ensue if the Pedley (1986) proposal were adopted (i.e. new names for more than 1000 species). A somewhat emotional argument, specifically Australian, against widespread taxonomic change is that the name *Acacia* is widely recognised here by the general public. It conveys images of trees and shrubs that are familiar in parks and gardens and in the wild, of a timber that makes beautiful furniture and turned products, and of a group of plants with a myriad of functions in everyday life. The alternative name *Racosperma* would not carry the same impact. Turnbull (2004) summarises a proposal from Orchard and Maslin (2003) that seeks the best of the old and the proposed new classifications and nomenclature. Their idea is to conserve the generic name *Acacia* for the largest subgenus, *Phyllodineae*. This would involve replacing the existing type species, *A. nilotica* (genus *Acacia*, subgenus *Acacia*), with a new type species, *A. penninervis* (genus *Acacia*, subgenus



*Phyllodineae*). The generic name *Acacia* would then be retained for the largest group, subgenus *Phyllodineae*, the species of which predominate in Australia. Other subgenera would become genera *Senegalia*, *Vachellia* or *Acaciella*. All this would mean fewer name changes than would be needed if the Pedley (1986) classification were adopted. A decision is likely in 2005 on how *Acacia* will be formally divided. We understand that the impending changes will inconvenience some of us but, at the same time, we appreciate the benefits that stem from creating out of the old, new genera that are monophyletic and of appropriate size (cf. Young 1996).

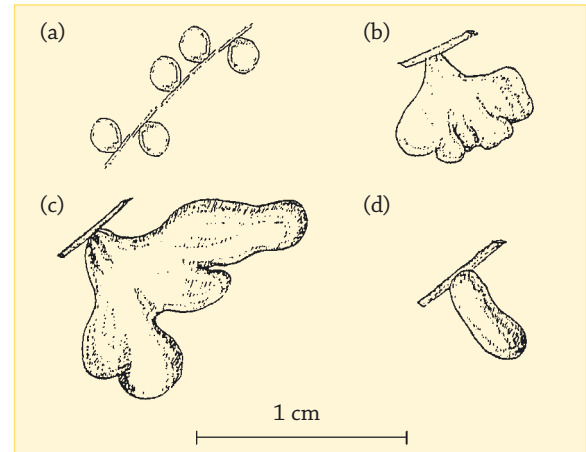
### ROOT AND NODULE MORPHOLOGY

Sprent et al. (1989) comprehensively reviewed the structure and function of the nodules of woody legumes, including acacias. As a general rule, N-fixing (effective) nodules of *Acacia* species are of the determinate (elongate) type. Notwithstanding, certain characteristics of acacia nodules are consistent with those of determinate (spherical) types (Lopez-Lara et al. 1993). It is easy to visually identify N-fixing nodules by the pink, leghaemoglobin-induced colour of their internal tissue. Those that are actively fixing N are usually cylindrical in shape, sometimes coralloid, occasionally multi-lobed. Ineffective nodules are globose (spherical) and small. Various nodule types are shown in Figure 2.

We sometimes observe the occurrence of so-called perennial nodules on acacia. Their shape is usually elongate with branching (see Fig. 2) and they appear to be partly lignified. We know of no investigation

of the perennial nodules of acacia. It seems unlikely that they are truly perennial. Perhaps their function is to provide the host plant with an immediate source of atmospheric N as soon as soil moisture becomes adequate following a prolonged dry period. This is analogous to the 'perennial' nodules of *Trifolium ambiguum* (Caucasian clover) which overwinter beneath snow, preserve a connection with the vascular system of the roots, and form new N-fixing tissue and commence N fixation at least two weeks before the appearance of new roots that can produce new nodules (Bergersen et al. 1963).

Corby (1971) was amongst the first to record nodule shapes (see also Fig. 2). He later expressed the opinion (Corby 1981) that nodule morphology might be useful in legume taxonomy. However, his idea has never been pursued.



**Figure 2.** Classification of the shapes of nodules (after Corby 1971) of acacias: (a) globose, (b) coralloid, (c) elongate with branching, (d) elongate, delicate



### 3. The bacteria

#### ACACIA RHIZOBIA IN NATURE

A painstaking search of the literature, undertaken by Allen and Allen (1981), recorded the occurrence of nodulation on less than 10% of acacia species. The figure is misleading, however, because it is likely that more than 90% of species simply had not then been examined for the presence of root nodules. Since 1981, nodulation has been reported for many more *Acacia* species (e.g. Kirkbride 2000; Sprent 2001b), and it now appears probable that all but a few acacias have the capacity to form nodules. Nevertheless, there is conclusive evidence that some species cannot nodulate.

There are three criteria that, taken together, constitute good evidence of non-nodulation: (i) inability to find nodules on the roots of the legume at field locations where it is endemic, (ii) failure of the legume to nodulate when it is grown, under benign conditions in the glasshouse, in soil taken from around roots of the plant in its natural habitat, and (iii) no nodulation of the legume in the glasshouse following its inoculation with a large collection, ideally hundreds of strains, of diverse rhizobia (J.I. Sprent, pers. comm.). The issue of non-nodulation in legumes, as with most negative data, is contentious. Notwithstanding, there are a number of reports of careful work on various

acacias failing to reveal nodules, e.g. *A. glomerosa* (Barrios and Gonzales 1971; de Faria and de Lima 1998), *A. greggii* (Martin 1948; Eskew and Ting 1978; Zitzer et al. 1996), *A. pentagona* (Corby 1974; Harrier et al. 1997), *A. polyphylla* (de Faria et al. 1987; de Faria and de Lima 1998) and *A. schweinfurthii* (Corby 1974; Harrier et al. 1997). We could find single, reliable records (Aronson et al. 1992; Moreira et al. 1992; Odee and Sprent 1992; de Faria et al. 1994; Zitzer et al. 1996; Masutha et al. 1997) of at least 12 other *Acacia* species that do not nodulate. It is probably no coincidence that the non-nodulating *Acacia* species all appear to be closely related as members of the subgenus *Aculeiferum*, ser. *Americanae* (de Faria and de Lima 1998). However, two species of the *Americanae* from Brazil, *A. bahaiensis* and *A. martii*, have been reported as nodulating plants (Allen and Allen 1981; Moreira et al. 1992; de Faria et al. 1994). Shaw et al. (1997), reporting on work with nodulating and non-nodulating tree legumes, drew attention to the existence of particular root exudates (*nod*-gene-inducing compounds) that played a role in initiating the symbiotic processes that led to nodule formation in the nodulating species but which were not present in the non-nodulating trees. Whether or not certain acacias fail to nodulate because they cannot produce these compounds is unknown.

This particular group of acacias (subgenus *Aculeiferum* ser. *Americanae*) is not endemic to Australia. Despite unconfirmed reports to the contrary cited by Allen and Allen (1981), we know of no Australian *Acacia* species (i.e. within the subgenera *Phyllodineae*, *Acacia* and *Aculeiferum*) that does not nodulate. Rhizobia are more common in soil immediately surrounding the root system (rhizosphere soil) than in non-rhizosphere soil (Robertson et al. (1995), working with *A. senegal*). It is our own experience with Australian acacias that, even when nodules cannot be found on the roots, rhizobia can be detected in the rhizosphere soil using the bait-plant technique (cf. Date 1980; Odee et al. 1995). There seems little doubt that the bacteria are almost as widely distributed in nature as the genus itself; see, e.g., papers by Miettinen et al. (1992) and Amora-Lazcano and Valdes (1992). Provided that the host is present, harshness of the soil environment appears immaterial. *Acacia* rhizobia occur in arid soils (Barnet and Catt 1991; Schulze et al. 1991; Dupuy and Dreyfus 1992), in dune sands (Barnet et al. 1985; Hatimi 1995), in surface soils and at depth (Dupuy et al. 1994), and sometimes at great depth — 34 metres (Dupuy and Dreyfus 1992).

In the field, the size of naturally occurring populations of acacia rhizobia varies considerably. Numbers as high as  $2.3 \times 10^5$  per gram of soil have been recorded (e.g. Odee et al. 1995). In many other situations, however, numbers may be very low or

absent (e.g. Thrall et al. 2001b). Where populations are small (<50 per g), rhizobial inoculation of acacia frequently results in enhanced N fixation (Turk et al. 1993). Turk et al. (1993) also report an unusual instance in which *A. mearnsii* responded to inoculation in the presence of >1000 naturally occurring rhizobia per gram of soil, but this finding is inconsistent with experience with other legumes, e.g. *Medicago* species (Brockwell et al. 1988) and soybean (*Glycine max*) (Thies et al. 1991a).

While high temperatures, high pH and high concentrations of salt limit nodulation and N fixation by rhizobia/acacia associations (see also below), there is substantial variability among strains in their ability to tolerate these conditions (Surange et al. 1997). We submit that such tolerance is widespread amongst acacia rhizobia and enables the organism to survive long periods of environmental extremes. For instance, it is our observation that, following rain in very arid parts of Australia, newly formed roots on *Acacia* species such as *A. tetragonophylla* quickly become nodulated, indicating a presence of rhizobia. The ensuing N fixation would provide the plant with a supply of atmospheric N and probably some ecological advantage. However, in such environments, the N supply is likely to be short-lived since N fixation will cease as soon as soil moisture stress becomes severe — e.g. Sprent (1971a,b); see also the later section dealing with soil moisture as an environmental factor limiting N fixation in the field.

## TAXONOMY OF ACACIA RHIZOBIA

The root-nodule bacteria (rhizobia<sup>3</sup>) that nodulate and fix N with legumes belong to at least six genera within the Rhizobiaceae: *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium* and *Allorhizobium*. These genera belong to three distinct phylogenetic branches within the  $\alpha$ -2 subclass of Proteobacteria. Quite recently, new genera of bacteria that nodulate legumes have been described. Sy et al. (2001) discovered a strain of a *Methylobacterium* sp. in nodules of *Crotalaria* spp. that constituted a fourth branch within the  $\alpha$ -2 subclass. Jaftha et al. (2002) characterised, as *Methylobacterium*, the pink bacteria that nodulate *Lotononis bainesii*. At much the same time, there were reports of species of *Burkholderia*, belonging to the  $\beta$ -subclass of the Proteobacteria, isolated from legume root-nodules. Moulin et al. (2001) reported the identification of a *Burkholderia* from nodules of *Aspalathus carnosa*, and Vandamme et al. (2002) noted that *B. tuberum* and *B. phymatum* nodulated the roots of tropical legumes. More recently, Ngom et al. (2004) isolated bacteria of the *Ochrobactrum* clade from the root-nodules of *A. mangium*.

Fred et al. (1932) distinguished two groups of root-nodule bacteria, the basic distinction being rate of growth: fast growers that acidify culture medium, and slow growers that do not acidify medium and tend to be associated with tropical legumes (Norris 1965). Both groups were considered

to be *Rhizobium*. It was not until Jordan (1982) that the new genus *Bradyrhizobium* was created to accommodate the slow growers. *Bradyrhizobium japonicum*, which nodulates soybean, is the type species of *Bradyrhizobium* and one of relatively few named species in the genus. There are other groups of strains that apparently belong to *Bradyrhizobium* but which have not been assigned to a species. It is customary to describe such strains as ‘*Bradyrhizobium* sp.’ followed, in parenthesis, by the name of the host genus, thus: *Bradyrhizobium* sp. (*Lupinus*). We accept this procedure for unnamed slow-growing strains of acacia rhizobia that belong to *Bradyrhizobium*, thus *Bradyrhizobium* sp. (*Acacia*), and for unnamed fast-growing strains of acacia rhizobia that belong to *Rhizobium*, thus *Rhizobium* sp. (*Acacia*). Other slow-growing acacia rhizobia include *Sinorhizobium saheli*; other fast-growing strains include *Mesorhizobium plurifarium*. Some strains of rhizobia that nodulate acacia may belong to still other as yet unnamed genera of the Rhizobiaceae and other families of bacteria. Barnett et al. (1985), for instance, recognised extra-slow-growing rhizobia that formed nodules on acacia. Also, Yonga (1996) implicated a new genus in nodulating acacias. She called it ‘*Pseudo-Bradyrhizobium*’. The taxonomy of the Rhizobiaceae as a whole is dealt with comprehensively by Young (1996), Young and Haukka (1996), Young et al. (2001) and Sawada et al. (2003), and neatly summarised by Sprent (2001b). The system of classification as defined by these authors, plus more recent additions, is shown in Table 3.

<sup>3</sup> In this review, we use the terms ‘rhizobia’, ‘root-nodule bacteria’ and ‘acacia rhizobia’ interchangeably to refer collectively to bacterial strains of genera of accepted legume symbionts (*Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium* and *Allorhizobium*), to strains of other genera not yet widely accepted as symbionts

(e.g. *Methylobacterium*, *Burkholderia* and *Ochrobactrum*), and to strains of other genera yet to be named and properly classified — e.g. ‘*Pseudo-Bradyrhizobium*’ (Yonga 1996).



**Table 3.** Nomenclature of rhizobia

Genus	Specific name	Hosts (not necessarily exclusive)	Additional references
<i>Rhizobium</i>	<i>R. leguminosarum</i>		Frank (1889)
	bv. <i>trifolii</i>	Species of <i>Trifolium</i>	
	bv. <i>viciae</i>	Species of <i>Pisum</i> , <i>Lathyrus</i> , <i>Lens</i> and <i>Vicia</i>	
	bv. <i>phaseoli</i>	Species of <i>Phaseolus</i>	
	<i>R. etli</i>		Segovia et al. (1993); Wang et al. (1999b)
	bv. <i>mimosae</i>	<i>Mimosa affinis</i>	
	bv. <i>phaseoli</i>	<i>Phaseolus vulgaris</i>	
	<i>R. gallicum</i>		Amarger et al. (1997)
	bv. <i>gallicum</i>	<i>P. vulgaris</i>	
	bv. <i>phaseoli</i>	<i>P. vulgaris</i>	
	<i>R. giardinii</i>		Amarger et al. (1997)
	bv. <i>giardinii</i>	<i>P. vulgaris</i>	
	bv. <i>phaseoli</i>	<i>P. vulgaris</i>	
	<i>R. galegae</i>		Lindstrom (1989)
	bv. <i>giardinii</i>	<i>Galega officinalis</i>	
	bv. <i>phaseoli</i>	<i>G. orientalis</i>	
	<i>R. hiananense</i>	<i>Desmodium sinuatum</i>	cited from Sprent (2001b)
	<i>R. huautlense</i>	<i>Sesbania herbacea</i>	Wang et al. (1998)
	<i>R. mongolense</i>	<i>Medicago ruthenica</i>	Van Berkum et al. (1998)
<i>R. tropici</i>	<i>Phaseolus vulgaris</i> , <i>Leucaena esculenta</i> , <i>L. leucocephala</i>	Martinez-Romero et al. (1991)	
<i>R. yanglingense</i>	<i>Amphicarpaea trisperma</i> , <i>Coronilla varia</i> , <i>Gueldenstaedtia multiflora</i>	Tan et al. (2001)	
<i>Bradyrhizobium</i>	<i>B. japonicum</i>	<i>Glycine max</i>	Jordan (1982)
	<i>B. elkanii</i>	<i>G. max</i>	Kuykendall et al. (1992)
	<i>B. liaoningense</i>	<i>G. max</i>	Xu et al. (1995)
	<i>B. yuanmingense</i>	Species of <i>Lespedeza</i>	Yao et al. (2002)
	<i>B. betae</i>	Unknown	B. Lafay and J.J. Burdon, unpublished data
	<i>B. canariense</i>	Unknown	B. Lafay and J.J. Burdon, unpublished data
	Several unnamed species of <i>Bradyrhizobium</i>	Genera of many species that are nodulated by slow-growing strains of rhizobia	

**Table 3.** (cont'd) Nomenclature of rhizobia<sup>a</sup>

Genus	Specific name	Hosts (not necessarily exclusive)	Additional references
<i>Sinorhizobium</i> <sup>a</sup>	<i>S. abri</i>	<i>Abrus precatorius</i>	Ogasawara et al. (2003)
	<i>S. indiaense</i>	<i>Sesbania rostrata</i>	Ogasawara et al. (2003)
	<i>S. meliloti</i>	Species of <i>Medicago</i> , <i>Melilotus</i> and <i>Trigonella</i>	Dangeard (1926)
	<i>S. medicae</i>	Species of <i>Medicago</i>	Rome et al. (1996a,1996b)
	<i>S. adhaerens</i>	Not known	Willems et al. (2003); Young (2003)
	<i>S. arboris</i>	<i>Acacia senegal</i>	Nick et al. (1999)
	<i>S. fredii</i>	<i>Glycine max</i> , <i>Cajanus cajan</i> and <i>Vigna unguiculata</i>	Scholla and Elkan (1984)
	<i>S. kostiense</i>	<i>Acacia senegal</i>	Nick et al. (1999)
	<i>S. kummerowiae</i>	Not known	cited from Young (2003)
	<i>S. morelense</i>	<i>Leucaena leucacephala</i> <sup>b</sup>	Wang et al. (2002)
	<i>S. saheli</i>	Species of <i>Acacia</i> and 'a number of other tree genera'	de Lajudie et al. (1994)
	<i>S. terangae</i>		de Lajudie et al. (1994)
	bv. <i>acaciae</i>	Species of <i>Acacia</i>	
	bv. <i>sesbaniae</i>	Species of <i>Sesbania</i>	
	<i>S. xinjiangense</i>	<i>Glycine max</i>	Peng et al. (2002)
<i>Mesorhizobium</i>	<i>M. loti</i>	Species of <i>Lotus</i> , <i>Anthyllis</i> and <i>Lupinus</i>	Jarvis et al. (1982)
	<i>M. amorphae</i>	<i>Amorpha fruticosa</i>	Wang et al. (1999a)
	<i>M. ciceri</i>	<i>Cicer arietinum</i>	Nour et al. (1994)
	<i>M. huakuii</i>	<i>Astragalus sinicus</i>	Chen et al. (1991)
	<i>M. mediterraneum</i>	<i>Cicer arietinum</i>	Nour et al. (1995)
	<i>M. plurifarium</i>	Species of <i>Acacia</i> , <i>Chamaecrista</i> , <i>Leucaena</i> and <i>Prosopis</i>	de Lajudie et al. (1998b)
	<i>M. septentrionale</i>	<i>Astragalus adsurgens</i>	Gao et al. (2004)
	<i>M. temperatum</i>	<i>A. adsurgens</i>	Gao et al. (2004)
	<i>M. tianshanense</i>	Various legumes including <i>Glycine max</i>	Chen et al. (1995)
	<i>Azorhizobium</i>	<i>A. caulinodans</i>	Nodulates stems and roots of <i>Sesbania rostrata</i>
<i>Allorhizobium</i>	<i>A. undicola</i>	<i>Neptunia natans</i>	de Lajudie et al. (1998a)
<i>Blastobacter</i>	<i>B. denitrificans</i>	<i>Aeschynomene indica</i>	Van Berkum and Eardly (2002)
<i>Burkholderia</i>	<i>Burkholderia</i> sp.	<i>Aspalathus carnosus</i>	Moulin et al. (2001)

**Table 3.** (cont'd) Nomenclature of rhizobia<sup>a</sup>

Genus	Specific name	Hosts (not necessarily exclusive)	Additional references
	<i>B. caribensis</i>	'Tropical legume(s)'	Vandamme et al. (2002)
	<i>B. phymatum</i>	'Tropical legume(s)'	Vandamme et al. (2002)
	<i>B. tuberum</i>	'Tropical legume(s)'	Vandamme et al. (2002)
<i>Devosia</i>	<i>D. neptunii</i> <sup>c</sup>	<i>Neptunia natans</i>	Rivas et al. (2002)
<i>Methylobacterium</i>	<i>Methylobacterium</i> sp.	<i>Lotononis bainesii</i>	Jaftha et al. (2002)
	<i>M. nodulans</i>	Species of <i>Crotalaria</i>	Sy et al. (2001); Jourand et al. (2004)
<i>Ochrobactrum</i>	<i>Ochrobactrum</i> sp.	<i>Acacia mangium</i>	Ngom et al. (2004)
<i>Ralstonia</i> <sup>a</sup>	<i>R. taiwanensis</i>	<i>Mimosa</i> spp.	Chen et al. (2001, 2003)

Source: earlier references from generally after Young (1996), Nick (1998), Van Berkum and Eardly (1998) and Sprent (2001b).

<sup>a</sup> The generic name *Ensifer* (Young 2003; cf. Willems et al. 2003) may have nomenclatural priority over *Sinorhizobium*. The generic names *Wautersia* (Vanechoutte et al. 2004) and *Cupriavidus* (Vandamme and Coenye 2004) have been proposed as alternative nomenclature for *Ralstonia*. At the time of updating this table (February 2005), these alternative names have not been generally accepted.

<sup>b</sup> Although the novel species *Sinorhizobium morelense* was isolated from nodules of *Leucaena leucocephala*, it did not form nodules when re-inoculated on to the host plant (Wang et al. 2002); however, a strain closely related to the novel strain was able to nodulate *L. leucocephala*.

<sup>c</sup> The specific name '*neptunii*' is tentative.

## DIVERSITY OF ACACIA RHIZOBIA

Lange (1961) was one of the first to recognise great diversity amongst rhizobia from native legumes growing in south-western Australia. The extent of that diversity has been confirmed by Marsudi et al. (1999) with the rhizobia isolated from *Acacia saligna* growing in the same general area. Lafay and Burdon (1998), using a molecular approach, identified similar diversity in the structure of rhizobial communities nodulating acacias growing in forests in south-eastern Australia.

Lawrie (1981, 1985) demonstrated that nodulation of Australian acacias was induced by both *Rhizobium* and *Bradyrhizobium*. Barnet and Catt (1991) investigated *Acacia* rhizobia from diverse localities in New South Wales. They obtained, from the arid zone,

strains described as typically *Rhizobium*, and from rain forest and coastal heathlands, other strains described as typically *Bradyrhizobium*. A third type, strains from alpine areas, was extra-slow-growing and was thought to represent another genus. Later, Barnet et al. (1985) isolated this third type from *A. suaveolens* and *A. terminalis* growing on coastal dune sand. At the time, Pedley (1987) also considered that three genera were responsible for acacia nodulation. Similar observations have been reported for the rhizobia of African acacias (Habish and Khairi 1970; Dreyfus and Dommergues 1981). Indeed, Dreyfus and Dommergues (1981) isolated both *Rhizobium* and *Bradyrhizobium* from nodules on the same tree. Even more extraordinary was the isolation of both genera from the same nodule taken from a root of *A. abyssinica* (Assefa and Kleiner 1998).

Characterisation of naturally occurring populations of acacia rhizobia, using morphological, biochemical, symbiotic, electrophoretic, chromatographic and molecular characters, has been reported by a number of investigators including Zhang et al. (1991), Amora-Lazcano and Valdes (1992), de Lajudie et al. (1994), Dupuy et al. (1994), Lortet et al. (1996), Haukka et al. (1996, 1998), Milnitsky et al. (1997), Nuswantara et al. (1997), Odee et al. (1997), Swelin et al. (1997), Zahran (1997), Khbaya et al. (1998), Vinuesa et al. (1998) and Marsudi et al. (1999). Frequently, these data have been employed to define relatedness among strains isolated from mixed soil populations, using various forms of pattern analysis. It is usual in these studies to distinguish several major clusters or groups (of identity). The ratio of the number of clusters to the total number of strains examined appears to fall in the range 1:10 to 1:20. In addition, there is invariably a number of individual strains that are unrelated to any of the others. At the strain level, such procedures are often used for individual strain identification, which is an essential tool for studying rhizobial ecology, inter-strain competitiveness and the success of inoculation.

The same procedures are also used at higher levels of taxonomic classification, viz. biovar, species and genus. For example, results from a thin-layer chromatography analysis of the *nod* factors synthesised by rhizobia from *Acacia* and *Sesbania* led Lortet et al. (1996) to propose that the two groups be named, respectively, *Sinorhizobium teranga* bv. *acaciae* and *S. teranga* bv. *sesbaniae*. Zhang et al. (1991), using numerical analysis of 115 characters, concluded that the rhizobia of *A. senegal* and

*Prosopis chilensis* were extremely diverse in physiological and biochemical features, as well as in cross-nodulation patterns. Haukka et al. (1996) used molecular technology to assess the diversity of rhizobia isolated from the nodules of *A. senegal* and *P. chilensis*. Sequence comparison indicated that one strain was closely similar to *Rhizobium haukuii* and that the others belonged to the genus *Sinorhizobium*. Similar degrees of diversity, found using different methods, were recorded by Amora-Lazcano and Valdes (1992) and Haukka and Lindstrom (1994). Taxonomic positions of rhizobia from *A. albida* were determined by Dupuy et al. (1994) with sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). Most strains belonged to eight clusters which contained representatives of *Bradyrhizobium japonicum*, *B. elkanii* and *Bradyrhizobium* sp. The same rhizobia were also characterised with the Biolog™ system. (Biolog™ = sole carbon source utilisation.) The results obtained from the two procedures were poorly correlated.

It is obvious that acacia rhizobia are diverse organisms. It is also clear that acacia species belonging to the same taxonomic section, individual species and sometimes the same tree often form nodules, and perhaps fix N, with bacteria from diverse taxonomic groups. Orderly classification of the rhizobia has been difficult, but recent procedural modifications are now leading to groupings of strains that are reproducible using different methods. For example, McInroy et al. (1998), working with 12 rhizobial isolates from African acacias and other tropical woody legumes, reported for the first time congruence between the results of Biolog™ analysis and genotypic fingerprinting.

## COMMENT ON THE TAXONOMY AND DIVERSITY OF ACACIA RHIZOBIA

Many investigations of the diversity of rhizobia contribute little towards a better understanding of the complexities of rhizobial taxonomy except to confirm what is already very well known, viz. that the field is very complex. One-off papers that use a small number of taxonomic tools to deal with a small collection of strains assembled from a small number of plants growing in a relatively small area are particularly unhelpful. We believe that the best prospect for elucidating the field lies in ongoing programs using a polyphasic approach and pooling the resources of several laboratories. Progress has been made where this has been done. For example, a group of strains of acacia rhizobia was assigned to 'gel electrophoretic cluster U' by de Lajudie et al. (1994). Characterisation of the group by electrophoresis of total cell protein, auxanographaphic tests, DNA base composition, DNA-DNA hybridisation, 16S rRNA gene sequencing, repetitive extragenic palindromic PCR, and nodulation tests gave de Lajudie and 11 co-workers (1998b) sufficient confidence to propose former cluster U as a new species, *Mesorhizobium plurifarum*, and to deposit a type strain in the LMG (Laboratorium voor Microbiologie, Universiteit Gent) collection of bacterial strains (see also Table 3).

It is still fair to say, as did Young (1996), that the taxonomy of the Rhizobiaceae is 'in a state of flux'. However, continuing rapid advances in the field (e.g. Sawada et al. 2003) make it seem likely that before long it will be possible to be more confident about the classification of acacia rhizobia.

## ROOT INFECTION AND NODULE FORMATION

As with many other legumes, most infection of acacias by root-nodule bacteria appears to take place via root hairs — e.g. *A. albida* (Gassama-Dia 1997); *A. senegal* (Rasanen and Lindstrom 1999) — even though acacia root hairs are often sparse (Rasanen et al. 2001). However, alternative routes of infection of some species, viz. wound (crack) infection — e.g. Allen and Allen (1940) and Chandler (1978) — and infection through intact roots — e.g. Dart (1977) and de Faria et al. (1988) — cannot be ruled out. Gassama-Dia (1997) noted that nodulation occurred promptly following inoculation of young seedlings. Rasanen and Lindstrom (1999) reported that the infection process was normal at relatively high root temperatures below 38°C but that nodule formation was retarded at 38° and 40°C and ceased altogether at 42°C.

However, some *Acacia* species — e.g. *A. fleckii*, *A. macrostachya* (Harrier 1995) — appear not to produce root hairs. Rhizobia probably enter such plants by infecting breaks in the root system formed by emergence of lateral roots. This mode of infection was reported first by Allen and Allen (1940) in *Arachis hypogea* (peanut). Some strains of rhizobia may enter their different hosts either by root-hair infection or by break infection (Sen and Weaver 1984). Likewise, certain host legumes — e.g. white clover (*Trifolium repens*) (Mathesius et al. 2000) — may utilise both means of infection. The subject of legume root infection leading to nodulation has twice been comprehensively reviewed by Sprent (1994b, 2001b).

*Acacia* species have distinctly fibrous root systems, particularly in the seedling stage. We know of no published results of measurements of the frequency on acacia roots of sites (foci) that are available for infection and subsequent nodulation by rhizobia. However, our own unpublished observations indicate that infection foci occur at high frequency. For example, we have noted in acacia forests that the fibrous mass of surface roots immediately beneath the layer of leaf litter is often nodulated in great abundance. Under less favourable conditions for nodule formation, Hogberg and Wester (1998) observed a substantial reduction in the fine root biomass of acacias planted on tractor tracks left behind as a consequence of logging. This was accompanied by reductions in both root nodulation and mycorrhizal infection.



Ben Bohlool

A rhizobial infection thread in a root hair of *Trifolium repens* (white clover). An identical phenomenon, an early stage in the processes leading to nodule formation, occurs in *Acacia* species. In acacias, infection threads often initiate from sac-like structures that themselves develop from the point of primary infection of the root hair by the rhizobia (Rasanen et al. 2001).

Strains of non-tumour-forming *Agrobacterium* lacking genes for N fixation have been isolated from nodules of several African legumes, including *Acacia* species (de Lajudie et al. 1999). The precise role, if any, of these organisms in the host/rhizobial symbiosis is not understood. It is possible that the *Agrobacterium* may sometimes act as a vector to assist the rhizobia in the early stages of root infection. There is no evidence for this proposition except that a comparable phenomenon has been postulated for the infection of pea (*Pisum sativum*) by *R. leguminosarum* bv. *viciae* (Van Rensburg and Strijdom 1972a,b).

## RHIZOBIAL PRODUCTS

There is little published work on those bacterial products of acacia rhizobia that might influence various aspects of the symbiotic system. The mechanisms are probably similar whatever the rhizobial species. For instance, Bhattacharyya and Basu (1992) showed that *Bradyrhizobium* isolated from *A. auriculiformis* induced the production of the auxin, indole acetic acid (IAA), from tryptophan in culture. Likewise, Kefford et al. (1960) detected tryptophan in the root medium of axenic cultures of *Trifolium subterraneum* (subterranean clover). The tryptophan was partially converted to IAA when the cultures were subsequently inoculated with *R. leguminosarum* bv. *trifolii*. It has long been considered that auxin has a role in the formation and growth of legume nodules (Thimann 1936, 1939).

Extracellular polysaccharides are natural products of the growth of rhizobia (e.g. Dudman 1976) including those in isolates from the nodules of *A. cyanophylla*

(Lopez-Lara et al. 1993, 1995) and *A. senegal* (Lindstrom and Zahran 1993). When some strains, including acacia rhizobia (J. Brockwell, unpublished data), are grown in culture medium, polysaccharide production may be copious. An involvement has been postulated (Dudman 1977) for polysaccharides in strain specificity, i.e. the ability of certain strains to infect some legumes but not others. Polysaccharide-based encapsulation of rhizobial cells (Dudman 1968) may be a mechanism for the survival of the bacteria when they are exposed to environmental stress while free-living in the soil.

Siderophore production by rhizobia may also be a survival mechanism and/or an aid to

competitiveness. It is known that the capacity of siderophores to sequester and bind iron molecules inhibits iron-dependent fungi that may otherwise parasitise or compete with the bacteria. Siderophore production has been recorded for the rhizobia of *A. mangium* (Lesueur et al. 1993; Lesueur and Diem 1997).

Gene products of rhizobial cells are the catalysts of the intimate processes involved in the regulation of nodule formation and nitrogen fixation. Understanding in this area has advanced rapidly (Vincent 1980; Kennedy et al. 1981; Caetano-Annolles and Gresshoff 1991; Dakora et al. 1993; Bladergroen and Spaink 1998).



Alison Jeavons

*Acacia decurrens*



## 4. The symbiosis

The ancestors of rhizobia may have been on Earth many millions of years before the legumes appeared (Sprent 2001a). While how and when the symbiosis between legumes and root-nodule bacteria evolved remains a mystery, it is a subject that has aroused much speculation (e.g. Raven and Sprent 1989; Young and Johnston 1989; Herendeen et al. 1992; Young 1993; Sprent 1994a; Soltis et al. 1995) and some controversy (e.g. Norris 1956, 1958, 1965; Parker 1957, 1968).

What is not in question is that legumes and rhizobia are not dependent on each other for their very existence. There are numerous authenticated instances of legumes that, like non-symbiotic plants, successfully complete their life cycles, including reproduction, without ever becoming nodulated or fixing N. Indeed, many species of the Caesalpiniaceae (Brockwell 1994; Sprent 1995, 2001a) and some members of the other legume families — e.g. the genus *Chaetocalyx* (Fabaceae) (Diatloff and Diatloff 1977) — do not nodulate at all. Likewise, there are records of the root-nodule organism surviving for long periods in the field in the absence of a host that it can nodulate (e.g. Bergersen 1970) or in dry soil in storage. As a rule, however, the symbiosis confers advantages on both partners.

The benefits to the micro-organism are pronounced. Thies et al. (1995) showed that populations of *Bradyrhizobium* in soil were substantially enriched as a result of cropping with a homologous legume. That is, growth of the legume stimulated multiplication of the rhizobia with which it formed nodules. In elegant experiments with *Glycine max* (soybean) and *Bradyrhizobium japonicum*, Reyes and Schmidt (1979) and Kuykendall et al. (1982) demonstrated that the majority of the population of rhizobia in the soil and/or in nodules formed on soybean roots were derived from organisms that had occupied nodules on the roots of the previous year's soybean crop.

The advantage of the symbiosis to the host is less pronounced. Nevertheless, the ability to access a source of N unavailable to non-symbiotic plants helps the legume to compete ecologically as a volunteer or a weed, or to produce agronomically as a crop or pasture plant. Because it is less dependent than the organism, the legume can perhaps be considered as the major partner of the symbiosis.

Humans have long been aware of the benefits of symbiotic N fixation, even if they did not understand the process. In the 12th century BC, for example, Theophrastus, a Greek philosopher, wrote about the re-invigorating effect of growing



legumes on exhausted soil — cited by Fred et al. (1932). The global amount of biological N fixation is a matter for conjecture. Estimates by Burns and Hardy (1975) and Paul (1988), augmented by Bunt (1988), suggest that the annual total approaches or exceeds 200 million tonnes. Much of this N is derived from terrestrial natural systems, agriculture and forestry. According to calculations made by Peoples et al. (1995a), symbiotic systems in arable land and permanent pasture account for 80–84 million tonnes. Nitrogen fixation by leguminous trees in forests — and by symbiotic non-leguminous trees, e.g. the *Casuarina (Alloacasuarina)/Frankia* association — would add to the estimate. Indeed the figure may be higher still, since the Peoples et al. (1995a) calculations probably underestimated the substantial quantity of fixed N partitioned in plant roots (Zebarth et al. 1991; McNeill et al. 1997; Khan et al. 2000; Unkovich and Pate 2000; Peoples and Baldock 2001).

Whatever the figure for global biological N fixation, probably about half is due to N fixation by legumes. A substantial component of that is contributed by agricultural legumes particularly where plant-nutrient deficiencies in the soil, other than N, have been corrected. A striking instance is the exploitation of the exotic annual self-regenerating legume, subterranean clover (*Trifolium subterraneum*), for the benefit of Australian pastoral enterprises and as the legume component of ley-farming systems (Puckridge and French 1983). Application of phosphorus, and sometimes minor elements, accompanied by sowing subterranean clover inoculated with effective *Rhizobium leguminosarum* bv. *trifolii*, provided conditions

suitable for abundant symbiotic N fixation (e.g. Morley and Katznelson 1965). The result, which is an example of what we mean when we speak of harnessing N fixation, was the establishment of 38 million hectares of pasture, containing at least 5% subterranean clover (Pearson et al. 1997), on previously N-deficient land.

One of the first leguminous trees to be utilised at least partly for its N-fixing ability was leucaena (*Leucaena leucocephala*). Leucaena has a long history as a shade tree for coffee (*Coffea* spp.) and cocoa (*Theobroma cacao*). Its forage value was first noted in Hawaii by Takahashi and Ripperton (1949) who reported annual dry matter production of 20–25 tonnes per hectare, and foliage N amounting to 400–600 kg per hectare. A direct outcome of this observation was the utilisation of leucaena as the leguminous component of pastures sown to provide grazing and browse for beef cattle in northern Australia (Griffith Davies and Hutton 1970) and elsewhere in the world (Vietmeyer 1978).

With the exception of some green manure plants such as Chinese milk vetch (*Astragalus sinicus*) and a few other herbaceous species noted by Giller and Wilson (1991), legumes are only rarely cultivated merely for their capacity to fix N. Nitrogen fixation is a secondary consideration. Clover is primarily a forage, leucaena is grown as a browse or a shade tree, other species yield food and fibre, and so on. *Acacia* species have manifold uses but their paramount product is wood. This is attested to, for instance, in the proceedings of an international conference held in Vietnam in 1997 (Turnbull et al. 1998a) dealing with plantings of Australian

acacias in various parts of the world. None of the 60 contributions to the workshop proposed that *Acacia* spp. be grown solely as a source of biological N. It is a bonus that a wood producer should also fix N. In this review, we show how that attribute might be exploited without affecting wood production.

## MEASUREMENT OF NITROGEN FIXATION

Effective management of biological N fixation ultimately relies upon a capacity to measure it accurately (Peoples and Herridge 2000). Descriptions and/or appraisals of the various methods for measuring N fixation are given in Chalk (1985), Shearer and Kohl (1986), Ledgard and Peoples (1988), Peoples and Herridge (1990), Danso et al. (1993), Herridge and Danso (1995) and Unkovich et al. (1997).

There are four principal methods: (i) the acetylene reduction assay (ARA) as a measure of nitrogenase activity which, in turn, is an index of N fixation; (ii) the xylem-solute method, which measures N-containing compounds that are products of N fixation and are carried from the root nodules to the shoots in xylem sap; (iii) the N-difference method, which measures the difference in N uptake between a N-fixing legume and a non-N-fixing control plant; and (iv)  $^{15}\text{N}$ -isotopic methods, which measure proportions of  $^{15}\text{N}/^{14}\text{N}$  in N-fixing legumes and non-N-fixing controls. There are two popular applications of  $^{15}\text{N}$ -isotopic techniques: (a) involving the use of artificial  $^{15}\text{N}$  enrichment of the soil, and (b) involving the use of natural enrichment (natural abundance) of  $^{15}\text{N}$  in the soil.

### The acetylene reduction assay (ARA)

The application of ARA for measuring N fixation in nodules of *Acacia* spp. is detailed by Hansen et al. (1987). ARA is a widely used diagnostic tool dating back to Dilworth (1966) and Hardy and Knight (1967). It is apt for measuring nitrogenase activity (N fixation) at an instant in time, particularly that of free-living diazotrophs growing in culture medium. Its application, especially to higher plants including legumes, however, is fraught with many pitfalls, as listed by Sprent (1969), Witty (1979), Van Berkum and Bohlool (1980), Van Berkum (1984), Boddey (1987), Giller (1987), Sloger and Van Berkum (1988) and Witty and Minchin (1988). One of these, an acetylene-induced decline in nitrogenase activity during assay, is demonstrated by Sun et al. (1992a) using ARA for estimating the nitrogenase activity in nodulated roots of *A. mangium*. We believe that ARA is generally unsuitable for quantifying N fixation (nitrogenase activity) in acacias and other leguminous trees, but is a useful qualitative measurement. Notwithstanding, we have cited a number of investigations that used ARA to quantify N fixation in the glasshouse, nursery and field.

### The xylem-solute method

Many tropical legumes transport most of the nitrogenous products of their N fixation as ureides. The greater the dependence of a plant on fixed N, the higher the proportion of ureides to nitrates plus amino compounds in the xylem sap. This characteristic can be exploited to assay the proportion of ureides in the nitrogenous compounds in bleeding or vacuum-extracted xylem sap and for constructing calibration curves for estimating

N fixation (McClure and Israel 1979; Herridge 1982). A recent paper (Herridge and Peoples 2002) suggests that quite accurate estimates of legume N fixation can be obtained by the ureide assay of a single sample of xylem sap. The fixed N of most other symbiotic legumes is transported from the nodules as amides. Assays have been similarly developed to measure the proportion of amides to other nitrogenous compounds in the xylem sap of such legumes (Peoples et al. 1986, 1987). The amide assay is less sensitive than the ureide assay.

Xylem sap extraction is more easily achieved with herbs than with woody species. Besides, a lethal sampling, which is more acceptable for crop plants than for trees, is needed for most efficient extraction of xylem sap. Acacias appear to export the nitrogenous products of their N fixation as two amides, asparagine and glutamine. Hansen and Pate (1987b) suggest that the amide (in xylem sap) technique is not satisfactory for estimating N fixation in the *Acacia* spp. found in Western Australian forests. If that is so, the same constraint is likely to apply everywhere.

### **The nitrogen difference method**

This is the simplest method. It is based on the principle that the difference in N uptake between an inoculated, nodulated legume and an uninoculated control represents the amount of N fixed. When applied under bacteriologically controlled conditions using N-free media or substrate in the laboratory or glasshouse (Brockwell et al. 1982), the method is

accurate and reliable, but is less so when used in the field. The difficulty lies in the selection of the control so that both N-fixing and non-N-fixing plants contain the same amounts of soil-derived N in their shoots. Differences between the two plant types in their capacities to extract and accumulate soil N almost invariably exist. Even when a non-nodulating isolate of the test legume is used as a control, field results may be unreliable (Boddey et al. 1984).

### **The $^{15}\text{N}$ isotopic methods**

These methods separate legume N into two fractions: (i) N originating from soil N, and (ii) N originating from atmospheric N.

#### *$^{15}\text{N}$ enrichment*

Almost all soils are naturally enriched with  $^{15}\text{N}$  compared with the ratio of  $^{15}\text{N}/^{14}\text{N}$  in atmospheric N. The level of natural enrichment of plant-available N in soil can be increased artificially by incorporation of a  $^{15}\text{N}$ -enriched nitrogenous salt. The use of methods involving soil augmentation with  $^{15}\text{N}$  to estimate N fixation has been comprehensively reviewed (e.g. Chalk 1985; Danso 1988). Provided that the N-fixing test plant and its non-N-fixing control are well matched, the technique gives a reliable estimate of the proportion of legume N derived from atmospheric N (%Ndfa), which is averaged over time. A major disadvantage is that applied N, particularly nitrate, may interfere with nodulation (cf. Tanner and Anderson 1964). The technique requires sophisticated instrumentation.

*Natural <sup>15</sup>N abundance*

The natural <sup>15</sup>N abundance method depends on natural enrichment of plant-available N in the soil with <sup>15</sup>N to provide the benchmark difference in <sup>15</sup>N/<sup>14</sup>N ratios between atmospheric N and soil N. Otherwise, the considerations are similar to those for <sup>15</sup>N enrichment techniques, except that certain limitations differ (Mariotti et al. 1983; Shearer and Kohl 1986). The major advantage of the natural abundance technique is that, because no pre-treatment with <sup>15</sup>N salt is required, it can be applied to existing experiments or to trees growing in plantations or forests.

Both of the <sup>15</sup>N isotopic methods are likely to underestimate total N fixation in legumes because they take no account of fixed N in underground plant parts. Recent research findings for pasture legumes (Zebarth et al. 1991; McNeill et al. 1997; Khan et al. 2000; Unkovich and Pate 2000; Peoples and Baldock 2001) indicate that 50% or more of the total N may be partitioned below-ground.

**CONSIDERATION OF PROCEDURES FOR MEASURING N FIXATION**

We are indebted to Peoples et al. (1989) from whom we have summarised procedures for measuring N fixation. Their monograph describes in substantial detail the various methods we have listed, with emphasis on application to field-grown legumes. They pay particular attention to the proper evaluation and interpretation of analytical data, to applications, to advantages and to limitations. In comparing the methods, they stress that there is no 'correct way' to measure N fixation.

Chalk and Ladha (1999) are critical of both <sup>15</sup>N-enriched and <sup>15</sup>N natural abundance isotope dilution methods, because of the non-uniform distribution of isotopic N through the soil profile whether the discrimination is natural or imposed by <sup>15</sup>N enrichment. The authors are suspicious of the reliability of reference plants used to benchmark the extent and variability of isotopic discrimination in the soil. The consequences of their somewhat gloomy appraisal can be moderated by selection of non-N-fixing reference plants with root geography as similar as possible to that of the N-fixing target plant.

There are, of course, in studies with shrubs and trees, other difficulties that limit the accuracy of estimating N fixation (Boddey et al. 2000a). They include perennial growth, seasonal and yearly variations in N assimilation (e.g. Ladha et al. 1993; Peoples et al. 1996), and large plant-to-plant differences in growth and nodulation which can occur within species and even within a single provenance (e.g. Burdon et al. 1999). Paparcikova et al. (2000) noted a further complication: in the Amazon jungle, there was little if any N fixation by the leguminous component of primary forest whereas, following clearing, those same tree legumes fixed N in secondary vegetation sites.

Conscious of the aforementioned constraints, we recommend two procedures for assessing N fixation in acacias. When measuring the symbiotic effectiveness of strains of rhizobia, or the response of acacia seedlings to rhizobial inoculation, or using the 'whole-soil' inoculation technique (Bonish 1979; Brockwell et al. 1988) to estimate the N-fixing



capacity of a mixed population of rhizobia in soil, it is feasible to work with N-free media and bacteriological control in tubes (Thornton 1930), pouches (Somasegaran and Hoben 1994), Leonard jars (Leonard 1944), paper roll tubes (Gemell and Hartley 2000), or open pots in the laboratory and/or the glasshouse (Bergersen and Turner 1970; Gibson 1980). Under these conditions, the N-difference method works well.

When measuring the amount and rate of N fixed by acacias grown as seedlings in a nursery, or as trees in a plantation or forest, the natural abundance technique appears most appropriate. The choice of tree(s) to be used as reference plant(s) is critical. Of course, they must be non-N-fixing. This is not usually a problem with temperate species but requires some caution with tropical non-legumes, because some of them may obtain up to 40% of their N requirements from associative N fixation (Boddey 1987). The reference species and the test plant should have similar growth rhythms. Attention must be paid to sampling procedures. Ideally, estimates of the absolute amount of Nfix (N fixation) and of %Ndfa (proportion of whole plant N, or shoot N, obtained by fixation of N from the atmosphere) should be based on the ratio of  $^{15}\text{N}/^{14}\text{N}$  of whole plant N or shoot N, not on subsamples of single leaves or other plant parts (Bergersen et al. 1988). While this is feasible for sampling seedlings grown in nursery containers, it is impossible for trees of the plantation or forest. Boddey et al. (2000b) provide a comprehensive appraisal of all aspects of the natural  $^{15}\text{N}$  abundance technique applied to the quantification of biological N fixation by woody perennials. While they draw

attention to the many pitfalls that might be encountered when using the technique, they suggest that, used prudently, it currently represents the best means of measuring symbiotic N fixation by woody legumes growing in the field.

### **NITROGEN FIXATION IN GLASSHOUSE AND NURSERY**

The results of several pot studies using a variety of methods of measurement have confirmed that acacias have the capacity for symbiotic N fixation. Sanginga et al. (1990) showed that fixation occurred in 13 provenances of *A. albida* although at lower rates than in leucaena (*Leucaena leucocephala*). There were differences between provenances both in Nfix and %Ndfa, and the two parameters were highly correlated. Using the acetylene reduction assay (ARA), Sun et al. (1992a) found that N fixation, measured as nitrogenase activity, in young seedlings of *A. mangium*, was linked to the respiration of the nodulated roots. *Acacia smallii* grown at elevated concentrations of  $\text{CO}_2$  fixed more N than plants grown at ambient  $\text{CO}_2$  (Polley et al. 1997). Pokhriyal et al. (1996) noted that nitrogenase activity in *A. nilotica* was highest during the long days of summer. This observation was complemented by Lal and Khanna (1993) who showed (in field studies) a decline in N fixation by *A. nilotica* during winter months. Nitrogenase activity in the nodulated roots of *A. mangium* increased following applications of phosphorus (P) (Sun et al. 1992b). Likewise, Ribet and Drevon (1996) found that low nodule nitrogenase activity associated with P deficiency was linked to reduced nodule growth. On the other hand, Vadez et al. (1995) concluded that *A. mangium*

seemed not to need high levels of P for growth and N fixation. In *A. albida*, nitrogenase activity decreased after the initiation of water deficit treatments (Dupuy et al. 1994). This finding is consistent for legumes generally (e.g. Sprent 1971a,b). Aronson et al. (1992) grew 40 legumes, mostly trees, in two Chilean soils. Acacias nodulated and grew better than non-acacias and grew more quickly than several species of *Prosopis*. However, the only evidence that N fixation was responsible for the enhanced growth of the *Acacia* species was the relationship between rate of growth and extent of nodulation. Ndoye et al. (1995) measured N fixation in *A. albida*, *A. raddiana*, *A. senegal* and *A. seyal*. Each species fixed N (see Table 4). The estimates obtained using different non-N-fixing trees as reference (control) plants were in reasonably good agreement. A high %Ndfa did not always lead to high Nfix. Michelsen and Sprent (1994) recorded %Ndfa values in *A. abyssinica* nursery stock in the range 5–47%.

These data make it clear that, grown in pots, all of the *Acacia* species examined are capable of symbiotic

N fixation. Not all of the investigations quantified the N fixed and, even if they had, it would not be sensible to extrapolate from pot culture to the field. Besides, there are numerous field studies, dealt with below, that have been undertaken for that purpose.

## NITROGEN FIXATION IN THE FIELD

### Qualitative evidence

Lal and Khanna (1996) reported N fixation (ARA) in field-grown *A. nilotica*, which stopped during winter. Hansen and Pate (1987a), on the other hand, found that N fixation in *A. alata* and *A. pulchella* was restricted to the moist months of winter and spring, and essentially ceased during summer and autumn periods of water stress. Tuohy et al. (1991) sampled leaves from trees in Zimbabwe. They found that leaf N content was consistently higher in nodulating tree legumes, including *A. nigrescens*, than it was in non-nodulating trees of the legume family Caesalpinaceae or in non-legumes. Similar but less striking data were obtained by Yoneyama

**Table 4.** Total nitrogen (Nfix) and proportion of total nitrogen obtained from N fixation (%Ndfa) calculated for four *Acacia* species with the <sup>15</sup>N enrichment technique using the non-N-fixing leguminous trees, *Parkia biglobosa* and *Tamarindus indica*, as reference plants.

<i>Acacia</i> species	Reference plant <i>P. biglobosa</i>		Reference plant <i>T. indica</i>	
	Nfix (g/plant)	%Ndfa	Nfix (g/plant)	%Ndfa
<i>A. albida</i>	0.4 b*	30.4 b	0.5 b	44.2 b
<i>A. raddiana</i>	0.5 b	58.1 a	0.6 b	66.8 a
<i>A. senegal</i>	0.4 b	27.2 b	0.5 b	41.6 b
<i>A. seyal</i>	1.6 a	59.7 a	1.9 a	66.7 a

Source: Derived from Ndoye et al. (1995).

\* In any one column, values with a common letter are not significantly different from one another ( $P > 0.05$ ).

et al. (1993) from leaf samples of trees, including *A. auriculiformis*, in Brazil and Thailand. Following a prescribed fire in a mixed *Eucalyptus* forest, N fixed by the understorey legumes, *A. melanoxylon* and *A. mucronata*, increased from near zero after 12 months to 26 and 57 mg per plant, respectively, after 27 months (Hamilton et al. 1993). This observation is contrary to the findings of Hansen and Pate (1987a) who recorded the best N fixation by *A. pulchella* and *A. alata* in the first year following a controlled burn.



Courtesy of CSIRO Forestry and Forest Products

Australian acacias are used for many purposes in many countries. This 10-year-old plantation of *Acacia melanoxylon* (Tasmanian blackwood) at Gwendique Estate, Zimbabwe, is intended for milling.

Robertson (1994) used data obtained from modelling levels of soil N in *A. senegal*/*Sorghum bicolor* rotations to argue that the N-fixing potential of acacias is less important than their ability to extract N from deep in the soil profile. An analogous argument was put for lucerne (*Medicago sativa*) (Gault et al. 1991). But it is now recognised that *M. sativa*, despite having access to deep-soil N

inaccessible to other plants, is a prolific fixer of N (e.g. Heichel et al. 1984; Wivstad et al. 1987; Hardarson et al. 1988; Brockwell et al. 1995b; Gault et al. 1995; Peoples et al. 1995b; Kelner et al. 1997; Bowman et al. 2004).

### Quantitative evidence

Investigating alley cropping systems, Sanginga et al. (1995) stated that *A. mangium* grown as hedgerows might fix as much as 100–300 kg N per ha per year and *A. albida* and *A. senegal* as little as 20 kg N per ha per year. However, these data were not tabulated in their paper. N fixation of only 5.4 g per tree (Nfix) was recorded for *A. caven* grown in a Mediterranean-type climate in Chile over a period of two years (Ovalle et al. 1996). Although fixed N as a proportion (%Ndfa) represented 85% of total N accumulation, N content was only 1.2% of total biomass production. By comparison, another tree legume, *Chamaecytisus proliferus* subsp. *palmensis* (tree lucerne), grown in companion plots produced 10 times as much total biomass, although Nfix and %Ndfa values were similar. May (2001), using natural  $^{15}\text{N}$  abundance, measured exceptional N fixation by *A. dealbata* at Tanjil Bren, Victoria, Australia (mean annual rainfall 1900 mm). Over a 5-year period, on land prepared by burning and at high stocking rates, the *A. dealbata* fixed more than 700 kg N per hectare; about 75% of the N was retained in the plant parts, with the remainder in the soil.

Further records of N fixation by acacias in the field are presented in Table 5.

NITROGEN FIXATION IN ACACIAS

**Table 5.** Values reported for *Acacia* nitrogen (N) fixation in the field

Species	N fixation		Plant part(s)	Method <sup>b</sup>	Period	Citation
	kg/ha	%Ndfa <sup>a</sup>				
<i>Acacia albida</i>		2	Leaves	$\delta^{15}\text{N}$ natural abundance		Schulze et al. (1991)
<i>A. tortilis</i>		12	Leaves	$\delta^{15}\text{N}$ natural abundance		As above
<i>A. hebeclada</i>		15	Leaves	$\delta^{15}\text{N}$ natural abundance		As above
<i>A. kirkii</i>		17	Leaves	$\delta^{15}\text{N}$ natural abundance		As above
<i>A. erioloba</i>		21	Leaves	$\delta^{15}\text{N}$ natural abundance		As above
<i>A. reficiens</i>		24	Leaves	$\delta^{15}\text{N}$ natural abundance		As above
<i>A. karroo</i>		25	Leaves	$\delta^{15}\text{N}$ natural abundance		As above
<i>A. hereroensis</i>		49	Leaves	$\delta^{15}\text{N}$ natural abundance		As above
<i>A. mellifera</i>		71	Leaves	$\delta^{15}\text{N}$ natural abundance		As above
<i>A. seyal</i>		63		$\delta^{15}\text{N}$ natural abundance		Ndoye et al. (1995)
<i>A. raddiana</i>		62		$\delta^{15}\text{N}$ natural abundance		As above
<i>A. pulchella</i>		9-37	Nodules	ARA <sup>b</sup>		Hansen and Pate (1987a)
<i>A. alata</i>		2-29	Nodules	ARA		As above
<i>A. holosericea</i> <sup>c</sup>		8-16	Nodules	ARA	6 months	Langkamp et al. (1979)
<i>A. holosericea</i>	6.4		Nodules	ARA	Annual	Langkamp et al. (1982)
<i>A. holosericea</i>	4-11	30	Whole plant	$\delta^{15}\text{N}$ enrichment and N difference	6.5 months	Cornet et al. (1985) <sup>d</sup>
<i>A. dealbata</i>	2-140		Whole plant and soil	$\delta^{15}\text{N}$ natural abundance	Per year for 5 years	May (2001)
<i>A. dealbata</i>	12-32		Nodules	ARA	Annual	Adams and Attiwill (1984)
<i>A. mearnsii</i>	200		Whole plant	N difference	Annual	Orchard and Darb (1956)
<i>A. mearnsii</i>	0.75		Nodules	ARA	Annual	Lawrie (1981)
<i>A. melanoxydon</i>	0.01		Nodules	ARA	Annual	As above
<i>A. paradoxa</i>	0.04		Nodules	ARA	Annual	As above
<i>A. oxycedrus</i>	0.12		Nodules	ARA	Annual	As above
<i>A. verniciflua</i>	32					Turvey et al. (1983)
<i>A. mangium</i>		20-90	Leaves	$\delta^{15}\text{N}$ natural abundance	19 months	Galiana et al. (1996)
<i>A. longifolia</i> var. <i>sophorae</i>	0.30		Nodules	ARA	Annual	Lawrie (1981)
<i>Acacia</i> sp.	52-66		Whole plant	$\delta^{15}\text{N}$ natural abundance		Peoples, Almendras and Dart <sup>e</sup>



**Table 5.** (cont'd) Values reported for *Acacia* nitrogen (N) fixation in the field

Species	N fixation		Plant part(s)	Method <sup>b</sup>	Period	Citation
	kg/ha	%Ndfa <sup>a</sup>				
<i>Acacia</i> sp.	51-81		Whole plant	$\delta^{15}\text{N}$ natural abundance		Dart and Almendras <sup>e</sup>
<i>Acacia</i> sp.	84-88		Whole plant	$\delta^{15}\text{N}$ natural abundance		Dart and Almendras <sup>e</sup>
<i>Acacia</i> sp.	34-67		Whole plant	$\delta^{15}\text{N}$ natural abundance		Dart and Almendras <sup>e</sup>
<i>Acacia</i> sp.	69		Whole plant	$\delta^{15}\text{N}$ natural abundance		Palmer <sup>e</sup>
<i>Acacia</i> sp.	56		Whole plant	$\delta^{15}\text{N}$ natural abundance		Palmer and Tatang <sup>e</sup>
<i>Acacia</i> sp.	59		Whole plant	$\delta^{15}\text{N}$ natural abundance		Palmer and Tatang <sup>e</sup>

<sup>a</sup> %Ndfa is the proportion (%) of total N in the plant (or plant part) derived from the atmosphere by symbiotic N fixation.

<sup>b</sup> ARA = the acetylene reduction assay.

<sup>c</sup> Originally identified as *Acacia pellita* O. Schwarz — Langkamp et al. (1979), then Corrigendum (1980).

<sup>d</sup> Trees grown in containers of 1 m<sup>3</sup> volume, not in the field.

<sup>e</sup> Unpublished data.

Measurements for other tree legumes are given in Table 6. The most consistent feature of the diverse measurements of N fixation by acacias is their inconsistency. Values ranged from 0 to 200–300 kg N per ha per year, but were generally at the lower end of that scale. Patterns of N fixation over time were sometimes contradictory. A remarkable discrepancy between the growth of acacias in the field and in the glasshouse was reported by Hansen and Pate (1987a). They compared symbiotic seedlings of *A. alata* and *A. pulchella* regenerating in a forest ecosystem with seedlings of the same species, inoculated with forest soil containing naturally occurring strains of acacia rhizobia, growing in N-free medium in the glasshouse. By 19 months, the glasshouse plants had gained 130–230 times more dry weight and had accumulated 110–160 times more total N than seedlings in the forest.

We do not believe that the variability in results from the field can be attributed solely to measurement

methodology. Perhaps the figure quoted by Orchard and Darb (1956) — 200 kg N fixed per ha per year — was on the high side of reality because the N difference method that they used has a tendency towards overestimation. It is not a simple matter, either, to reconcile the promising values for acacia N fixation obtained in glasshouse and nursery with the generally low values from the field. In the next section, we consider factors that might affect N fixation performance of field-growing acacias. Environmental factors and symbiotic factors are considered separately. Symbiotic factors are deemed to include relationships between acacias and mycorrhizae as well as associations between acacias and rhizobia. Later, we will also consider what implications those factors might have in relation to optimisation and exploitation of N fixation in acacias in order to enhance the N nutrition and vigour of the tree, to conserve soil N and to contribute to the sustainability of forest and plantation ecosystems.

**Table 6.** Estimation of nitrogen (N) fixation in leguminous trees other than species of *Acacia*

Genus	N fixation		Period	Citation
	kg/ha	%Ndfa <sup>a</sup>		
<i>Aotus ericoides</i>	1		Annual	Lawrie (1981)
<i>Albizzia</i>	94	60	Annual	Liya et al. (1990)
<i>Albizzia</i>		55		Peoples, Almendras and Dart <sup>b</sup>
<i>Calliandra</i>	11	14	90 days	Peoples and Palmer <sup>b</sup>
<i>Gliricidia</i>	108	72	Annual	Liya et al. (1990)
<i>Gliricidia</i>	13		Annual	Roskoski et al. (1982)
<i>Gliricidia</i>	99	75	Annual	Peoples and Palmer <sup>b</sup>
<i>Gliricidia</i>		60	Annual	Peoples and Ladha <sup>b</sup>
<i>Inga</i>	35		Annual	Roskoski (1981)
<i>Leucaena</i>	110		Annual	Hogberg and Kvarnstrom (1982)
<i>Leucaena</i>	296–313	58–78	3 months	Zoharah et al. (1986)
<i>Leucaena</i>	288–344	34–39	6 months	Sanginga et al. (1989b)
<i>Leucaena</i>		59–100		Yoneyama et al. (1990)

Source: derived from Peoples and Craswell (1992), Khanna (1998) and unpublished data.

<sup>a</sup> %Ndfa is the proportion (%) of total N in the plant (or plant part) derived from the atmosphere by symbiotic N fixation.

<sup>b</sup> Unpublished data.

## ENVIRONMENTAL FACTORS AFFECTING NITROGEN FIXATION IN THE FIELD

### General considerations

A principle of limiting factors states that ‘the level of crop production can be no higher than that allowed by the maximum limiting factor’. If a similar principle applied to symbiotic N fixation by acacias, it would follow that the level of N fixation would be strongly linked to the physiological state of the host tree. However effective an association between an acacia and a rhizobial strain might be, it cannot realistically be expected to express its full potential for N fixation if limiting factors such as nutrient deficiency or excess, salinity, unfavourable soil pH, soil microbiology, and/or insufficient soil

moisture impose limitations on the host (cf. Thies et al. 1991a,b; Peoples et al. 1995a). Giller and Wilson (1991), dealing with N fixation by leguminous trees and shrubs as well as by agricultural legumes in tropical settings, presented a comprehensive review of environmental constraints to N fixation.

In the previous section, we showed that acacias fared poorly in N fixation by comparison with other leguminous trees (Tables 5 and 6). However, a direct comparison of the two groups may not be valid. Nitrogen-fixation studies of tree legume genera such as *Calliandra*, *Gliricidia* and *Leucaena* were mostly done on trees planted in hedgerows, often fertilised and inoculated with effective root-nodule bacteria, and intended for periodic harvesting and grazing

of foliage. On the other hand, much of the work on acacias was done in natural habitats—savannas, sand dunes, mixed-species forests.

An apt analogy is subterranean clover (*Trifolium subterraneum*), which is a legume component of some 38 million hectares of pasture in southern Australia and a prolific fixer of N. It is an insignificant species in its native habitat on the mainly acid, phosphorus-deficient soils of the Mediterranean basin. Indeed, for 50–80 years after its accidental, but continuing, introduction into Australia, it remained a plant of little importance. It was not until the early 1900s, when superphosphate began to be used, and when other nutrient element deficiencies were corrected later, that subterranean clover responded and began its rise to prominence as a pasture plant (Morley and Katznelson 1965). Even then it flourished only in soils where effective strains of rhizobia occurred. It is apparent that the success of subterranean clover in Australia is a consequence of good agronomic management. We submit that efficient silvicultural management will be the key to realising the full potential of acacias for production of wood and symbiotically fixed N. However, before that potential can be exploited, the factors that currently limit productivity and N fixation must be defined.

### Soil moisture

Habish (1970) regarded 15% soil moisture as optimal for growth and nodulation of *Acacia* species generally. Information about the relationship between N fixation in acacias and soil moisture is contradictory. Schulze et al. (1991) used natural  $^{15}\text{N}$

abundance to measure the proportion of leaf N due to N fixation in arid environments (30–400 mm annual rainfall) in Namibia. They recorded values ranging from 2% Ndfa for *A. albida* to 71% Ndfa for *A. mellifera* (see Table 4). They were unwilling, however, to attribute their results solely to N fixation, speculating that deep-rooted species may have accessed soil that was highly enriched with  $^{15}\text{N}$  (cf. Virginia et al. 1989). It was not mentioned whether the roots of the trees also had access to groundwater. Robertson (1994), modelling data obtained from an arid-zone system, concluded that *A. senegal* had limited potential for symbiotic N fixation. Newton et al. (1996) regarded out-planted *A. tortilis* subsp. *spirocarpa* as a tree with a relatively high water-use efficiency that, in some situations, might be further improved by rhizobial inoculation. Barnet et al. (1985), working with *A. longifolia* var. *sophorae*, *A. suaveolens* and *A. terminalis* growing on sand dunes, found that N fixation maximised in late autumn when moisture was readily available and declined in late spring and early summer as moisture became limiting and nodule senescence increased. Seasonal changes in nodulation that lead to substantial variations in rates of N fixation are characteristic of Australian native legumes and have been recorded over a long period for a number of species across a wide geographic range (e.g. Beadle 1964; Langkamp et al. 1981, 1982; Lawrie 1981; Monk et al. 1981; Hingston et al. 1982; Hansen and Pate 1987a,b; Lal and Khanna 1996). Deans et al. (1993) worked with a soil from Sudan that contained large populations of acacia rhizobia but in which *A. mellifera* trees did not form nodules. They attributed the lack of nodulation to low soil moisture.

There seems no reason why the effect of moisture stress on N fixation by acacias should be any different from what was demonstrated in the classical work of Sprent (1971a,b) working with the nodules of soybean. Whenever and wherever water is deficient, legume N fixation will be impaired. Should the deficiency be severe, N fixation will cease. It is also probable that acacias, like other legumes, will shed nodules (Sprent 1971a; Sheaffer et al. 1988) as well as other below-ground parts during periods of great moisture stress.

### Soil temperature

There appears to be little recent published information on the effects of temperature on acacia nodulation and N fixation. However, there is no reason to suppose that influences of low and high temperatures on acacia symbioses would differ very much from temperature effects on the symbioses of forage legumes (e.g. Gibson 1963, 1969, 1971; Harding and Sheehy 1980).

Habish (1970) found that *Acacia* species produced effective nodules at temperatures up to 35°C, which he considered 'the highest temperature for nodulation so far recorded'. Rasanen and Lindstrom (1999) studied the effects of high temperature on rhizobial infection of acacia root hairs, which is an early step in the processes leading to nodule formation. They found that infection and nodulation were normal at (high) temperatures below 38°C but that nodulation was reduced at 38° and 40°C and ceased completely at 42°C.

Many acacias grow in hot climates where, at certain times of the year, surface soil temperatures are high enough to prevent N fixation altogether (cf. Gibson 1971). However, it is distinctly possible that deep-rooted legumes such as acacias may escape extremes of environment by forming the bulk of their nodules (and fixing most of their N) at depth, where conditions are more benign. An analogous circumstance has been reported from a hot climate in Australia. In a 3-year-old stand of lucerne (*Medicago sativa*), more than 99% of the total soil population of lucerne rhizobia (*Sinorhizobium meliloti*) congregated at 30–60 cm depth in the soil profile; presumably, the majority of nodulation and N fixation took place in the same vicinity (Evans et al. 2005).

### Light

There is substantial evidence that low levels of light restrict the growth of legumes [references cited by Sprent (1999)] due, at least in part, to deleterious effects on N fixation. This may be of significance for acacias growing as forest understorey. In Australia, acacias are often the dominant recolonising species following bushfire and may fix abundant N during this phase (e.g. Adams and Attiwill 1984; Hansen and Pate 1987a,b). In the following phase of succession, as species of *Eucalyptus* become dominant, the acacia understorey becomes sparse and obviously less effective in fixing N. Competition for light may contribute to the condition. Roggy and Prevost (1999) recorded nodulation of both shade-tolerant and shade-intolerant legume trees growing in tropical forests but did not measure relative levels of N fixation.



## Nutrients

There are several comprehensive reviews of the nutrient needs of legumes generally, both dependent on and independent of biological N fixation (e.g. Vincent 1965; Munns 1977; Smith 1982; O'Hara et al. 1988; Giller and Wilson 1991). Information on the requirements of acacias, on the other hand, is sparse. For symbiotic legumes, the mineral requirements of the rhizobia, the infection process, nodule development and nodule function are usually less than for the plant itself. But there are important exceptions — references cited by Brockwell et al. (1995a). For instance, for nitrogenase (N-fixing) activity, molybdenum and cobalt are needed far in excess of other plant requirements (Evans and Russell 1971). Also, evidence is accumulating (McLaughlin et al. 1990) of a specific effect of P on the growth and survival of rhizobia and their capacity for nodulation and effective N fixation (Singleton et al. 1985).

Critical foliar levels for the nutrient elements that may limit the growth of acacias are yet to be determined (Simpson et al. 1998). However, while it is quite obvious that there are substantial differences in nutritional requirements between *Acacia* species, it is also clear that acacias are less demanding of nutrients than many agricultural legumes. This character is exploitable. For instance, vast areas of degraded, infertile, *Imperata* grasslands in Indonesia are scheduled for reforestation with *A. mangium* (Turnbull et al. 1998b). This is not to say that acacias are immune to mineral deficiency. Dell (1997) found boron and iron deficiencies, and nickel toxicity, in *A. mangium* growing on very infertile acid soils in parts of China, the Philippines and Indonesia.

The literature is somewhat confusing about the response of acacias to fertiliser. Although acacias are generally adapted to soils of low fertility, many (tropical) species respond to the application of fertilisers (e.g. Cole et al. 1996). In particular, response to added phosphorus (P) is common, and benefits have also been obtained by applying micro-nutrients and mineral N (Mead and Miller 1991; Goi et al. 1992; Turvey 1995; Cole et al. 1996). Generally, early growth of nursery seedlings is increased by adding mineral N as a starter, but low levels of nitrate depress nodule formation by *A. auriculiformis* (Goi et al. 1992). Plantation trees respond better to fertiliser added at planting than to applications after canopy closure (Otsamo 1996). In *A. mangium*, there is a link between low P and poor N fixation (Mead and Speechly 1991) as there is with herbaceous crop and pasture legumes (cf. Vincent 1965). On the other hand, Ryan et al. (1991) reported relatively small, albeit significant, responses to the application of a complete mineral fertiliser including N by newly outplanted *A. neriifolia*, *A. cincinnata*, *A. leptocarpa*, *A. mangium*, *A. crassicaarpa* and *A. plectocarpa*. A curious side-effect was that survival of seedlings was depressed significantly by addition of fertiliser. This appeared to be due primarily to P, but trace elements were implicated also.

Lesueur and Diem (1997) showed that the *A. mangium/Bradyrhizobium* association had a high and ongoing requirement for exogenous iron to promote nodulation and maintain N fixation. Their results were interpreted to mean that *A. mangium* lacked mechanisms to store phytoferritins in its tissues.

In considering the sometimes conflicting results of investigations relating to nutrition, we concur with the views of Dommergues (1982) who concluded that the productivity of N-fixing trees and their ability to fix N were dependent, amongst other things, on nutrient status. Likewise, Jordan (1985) considered that nutrient deficiency was the major factor limiting forest productivity in the humid tropics.

Experimental acacia plantations in Australia are typically (Mitchell 1998; Searle et al. 1998) but not always (Bird et al. 1998) supplied with superphosphate and sometimes nitrogenous fertiliser at planting and/or at 12 and 24 months thereafter. By contrast, acacias established by direct seeding in land reclamation undertakings (see below) in southern Australia are rarely, if ever, supplied with mineral fertiliser; they are, however, inoculated with strains of rhizobia to provide a source of biological (fixed) N.

### Nitrate

Inhibition of nodulation and N fixation by nitrate ( $\text{NO}_3^-$ ) is well known (Vincent 1965; Peoples and Herridge 1990). The processes are not fully understood, although several hypotheses have been proposed (e.g. Tanner and Anderson 1964; Munns 1968; Dazzo et al. 1981). There are serious implications for leguminous field crops and their capacity to form effective symbioses (e.g. Herridge et al. 1984; Bergersen et al. 1985; Thies et al. 1991b). Indeed, Brockwell et al. (1995a) concluded that it was unwise to plant a legume into soils containing significant amounts of available  $\text{NO}_3^-$ .

It is very likely that  $\text{NO}_3^-$  also inhibits the nodulation and N fixation of *Acacia* species. Turk et al. (1993) considered that plant-available N in soil used in pot experiments reduced the response to inoculation by *A. auriculiformis*, *A. mangium* and *A. mearnsii*. The degree of inhibition seems bound to vary with circumstance. Toky et al. (1994) observed that application of urea reduced nitrogenase activity in nodulated *A. nilotica* without any corresponding effect on the extent of nodulation. Likewise, Thakur et al. (1996) found that short-term (5–15 days) application of nitrate to seedlings of *A. catechu* had no effect on nodulation. In plantation forests, establishment of acacia seedlings usually follows land preparation procedures that result in release of substantial amounts of  $\text{NO}_3^-$  from organic sources. The presence of such N is ephemeral due to leaching and other losses, and the element is soon unavailable to the developing seedlings. Therefore, fertiliser N is often applied at or soon after planting (e.g. Searle et al. 1998; Mitchell 1998). We believe that outplanting *Acacia* seedlings that are well nodulated and actively fixing N would remove any requirement for N fertiliser at planting.

In natural forest ecosystems and long-established plantation systems with a substantial component of N-fixing trees, leaf litter on the forest floor is relatively rich in organic N. As this is broken down, the soil profile immediately beneath the litter layer is likely to become enriched in plant-available N as  $\text{NO}_3^-$ . Due to higher rates of N cycling, N is more readily available in natural (tropical forest) ecosystems than in anthropogenic systems (e.g. Neill et al. 1999; Rhoades and Coleman 1999; Veldkamp et al. 1999; Paparcikova et al. 2000).



This has implications for the level of N fixation achieved by those N-fixing trees whose general nutrition is heavily reliant on surface-feeder roots. It also has significant implications for the N status of similar non-N-fixing trees or other species that might be used as reference plants as part of the natural  $^{15}\text{N}$  abundance technique for measuring symbiotic N fixation (Boddey et al. 2000b).

### Heavy metals

There is little doubt that the introduction of heavy metals by application of sewage sludge or spoil from mining operations reduces the size and diversity of populations of soil microflora, including rhizobia (e.g. McGrath et al. 1988; Chaudri et al. 1992, 1993; Angle et al. 1993; Dahlin et al. 1997; Giller et al. 1999). It has also been suggested that heavy metals may impair the effectiveness of N-fixing populations (Smith 1997). Those of the reports that deal with rhizobia focus mostly on *R. leguminosarum* bv. *trifolii*.

It has been argued that the extent of heavy metal impairment of microfloral populations is a function of soil pH and organic matter (Brendecke et al. 1993; Pepper et al. 1994), but there has been much debate on this (Pepper et al. 1994; Witter et al. 1994a,b; McGrath and Chaudri 1999).

We are unaware of any published information on effects of heavy metals on the rhizobia of acacias. However, some workers apparently take the view that *Acacia* species are more sensitive to heavy metals than are their rhizobia. For instance, Zhang et al. (1998) recommended screening lines of

*A. auriculiformis*, but not their rhizobia, for tolerance to zinc as a prerequisite for using the species for revegetation of zinc-contaminated land.

### Landfill gas

In Hong Kong, *A. confusa* and *Leucaena leucocephala* are widely used for revegetating landfill areas. Gaseous emissions from the landfill include oxygen, methane, carbon dioxide and acetylene. Prolonged exposure to landfill gases suppressed the growth and N fixation of these species (Chan et al. 1998).

### Soil salinity

A number of *Acacia* spp. tested in the field have been shown to have varying degrees of tolerance of saline soils (Aswathappa et al. 1986; Marcar et al. 1991a, 1998). Amongst the most tolerant are *A. ampliceps*, *A. nilotica*, *A. redolens*, *A. saligna* and *A. stenophylla*. There are differences between provenances of the same species (Craig et al. 1991; Marcar et al. 1991b, 1998).

Marcar et al. (1991a) demonstrated in sand-culture solution augmented with three levels of sodium chloride (NaCl) that *A. ampliceps*, *A. auriculiformis* and *A. mangium*, in that order, were progressively less tolerant of salinity. Sodium chloride concentrations of 400 mM markedly affected shoot dry matter, nodule number and nodule development in all three species. Tree species with artificially high levels of foliar N, induced by applications of ammonium nitrate, generally had lower shoot concentrations of sodium and calcium than

inoculated plants wholly dependent on symbiotic N. Otherwise, survival, damage symptoms and relative growth reduction as salt concentrations increased were similar for both normal and high N treatments.

Zhang et al. (1991) considered that rhizobia from the nodules of trees, including acacias, growing in Sudan had a high tolerance of salt. A strain isolated from *A. farnesiana* tolerated salt concentrations up to 5% (Surange et al. 1997). Two of 35 rhizobial isolates from *A. nilotica* grown in culture medium tolerated up to 850 mM NaCl (Lal and Khanna 1994). However, when those two strains were inoculated on to *A. nilotica* grown under saline conditions, they lost at least 75% of the N-fixing (nitrogenase) activity that they had exhibited when grown in combination under non-saline conditions. In glasshouse experiments, Zou et al. (1995) used two strains of rhizobia, one salt-tolerant and one

salt-sensitive, to inoculate *A. ampliceps* grown at increasing concentration of NaCl. Indices of N fixation — nodule number, acetylene reduction, N content per plant, plant growth — declined as salt concentration increased, but the decline was less where the *A. ampliceps* plants had been inoculated with the salt-tolerant strain than where they had been inoculated with the salt-sensitive strain. Craig et al. (1991) worked with two rhizobial strains isolated from *A. redolens* growing in saline areas and one each from *A. cyclops* and *A. saligna* growing in non-saline soils. All four were equally tolerant of buffered culture medium to which 300 mM NaCl had been added. Three of the strains, including both from the saline areas, when combined with a highly salt-tolerant provenance of *A. redolens* formed associations that did not differ in symbiotic characteristics irrespective of NaCl concentration up to 160 mM. On a less salt-tolerant provenance



Peter Thrall

Severely salted landscapes cannot be rehabilitated but their expansion can be contained by revegetation around their perimeters. Salt-tolerant species of *Acacia* are apt for this purpose.



of *A. redolens*, and on *A. cyclops*, the infectivity and N-fixing effectiveness of the strains fell as the external salt concentration increased. Cantrell and Linderman (2001) reported that effects of soil salinity on two non-legumes were ameliorated by pre-inoculation with arbuscular mycorrhizal fungi.

It appears from these investigations that the acacia root-nodule organism is less sensitive to external NaCl concentration than the acacia plant, and that the acacia symbiosis is at least as sensitive as the acacia plant. We concur with Bala et al. (1990) that it is sensible to use effective, salt-tolerant strains of rhizobia to inoculate acacia species intended for planting on saltlands. Under these conditions, one can be confident that, provided that the plant survives, so too will the bacteria.



Courtesy of CSIRO Forestry and Forest Products

Many acacias are substantially tolerant of salinity (Aswathappa et al. 1986). One such species is *Acacia stenophylla* (Eumong or river cooba). This fine specimen of *A. stenophylla* is growing on a saline sandy soil near Oodnadatta, South Australia.

### Soil reaction — acidity

There is scant literature on acacia tolerance of soil acidity. This may be because there is a complex of factors involved in soil acidity, so that screening plants for tolerance of acidity is more complicated than screening them for salt tolerance. Habish (1970) considered that soil reaction affected acacia nodulation more than it affected plant growth. At  $\text{pH}_{\text{water}} 5.0\text{--}5.5$ , nodulation was absent. Lesueur et al. (1993) recognised that *A. albida* and *A. mangium* plants, in contrast to acacia rhizobia, might be affected by acidity (soil pH 4.5), depending on plant provenance. Although aluminium toxicity in agricultural species is a common phenomenon in acid soils (e.g. Delhaize and Ryan 1995; Ma et al. 2001), Lesueur et al. (1993) could detect no effect of high concentrations of aluminium (100 mM  $\text{AlCl}_3$ ) on the growth of *A. albida* or *A. mangium*. They nevertheless considered that the ability of acacias to tolerate soil acidity should be taken into account when screening *Acacia/Bradyrhizobium* combinations for use in afforestation trials. Searching for acid-tolerant woody legumes suitable for alley cropping, Kadiata et al. (1996) eliminated *A. auriculiformis* as a candidate. *Acacia mangium* had a high rate of N fixation (measured as acetylene reduction) in acid soil in Costa Rica (Tilki and Fisher 1998). Snowball and Robson (1985) observed that *A. signata*, a species adapted to acidic soil, responded to application of lime. This was thought to be due to alleviation of manganese toxicity. Ashwath et al. (1995) used glasshouse experiments with two soil types to rank 36 symbiotic *Acacia* species according to acid tolerance. Ranking differed somewhat with soil type. *Acacia julifera*, *A. aneura*, *A. difficilis* and *A. tumida* were ranked as highly tolerant or

tolerant on both types. The highly salt-tolerant species, *A. stenophylla*, was very sensitive to acidity. Other results from the experiments indicated that acacias are likely to benefit from inoculation with effective rhizobia, particularly when grown on acidic soils. Kang et al. (1998) observed that most acacia rhizobia are sensitive to acidity but that strongly acid-tolerant strains do exist.

### Soil reaction — alkalinity

Tolerance of alkaline conditions by certain acacia rhizobia is also common and occasionally quite extraordinary. For instance, a strain isolated from *A. farnesiana* was well adapted to grow at pH 12.0 (Surange et al. 1997). Habish (1970), on the other hand, speculated that the alkaline reaction of soils in northern Sudan might be a factor in reducing (nodulation and) plant growth of acacias in that region.

### Cluster roots

Many plants, including acacias, have cluster (proteoid) roots, which are one of several means by which plants cope with inhospitable environments (Sprent 1999). Cluster roots may be important in N acquisition, supplementing N fixation (Sprent 1995), as well as facilitating uptake of other nutrients — e.g. iron (Waters and Blevins 2000) — and soil moisture (Sprent 1995).

### Soil fauna

The density of soil fauna is sometimes regarded as an index of 'soil health'. Based on studies of

Collembola (non-insectan arthropods, springtails) in forest soils, Pinto et al. (1997) concluded that the presence of acacias contributed to increases in the population densities of soil fauna. Synergistic phenomena such as these are probably commonplace and are mediated also by non-leguminous trees (e.g. Hansen 1999).

### Parasites and pests

Like most other plants, acacias are susceptible to predation by nematodes, e.g. *Meloidogyne* spp. (Duponnois et al. 1997a,b). The attacks are generally directed at the roots, and it seems reasonable to suppose that the symbiotic system would sustain collateral damage. Robinson (1961) and Taha and Raski (1969) report nematode invasion of the nodules of agricultural legumes. Duponnois et al. (1997a) observed that *M. javanica* infestation reduced N fixation by *A. mangium* and *A. holosericea*. *Acacia tumida* and *A. hilliana*, on the other hand, fixed more N in the presence of the nematode.

Like other plants, acacias are subject to infestation by mistletoes (e.g. *Olex* spp.), hemiparasites (e.g. *Santalum* spp.) and various strangler vines. Ku et al. (1997) and Tennakoon et al. (1997a,b) investigated some consequences of the parasitic association between nodulated *A. littorea* and *Olex phyllanthi*. Parasitism had no effect on the total increment of N fixed. However, partitioning within the acacia plant of the products of N fixation was disturbed, which led to decreased shoot biomass and total plant N but increased root growth. About 9% of the haustoria of the mistletoe was attached directly to the root nodules of the



acacia which almost certainly provided the parasite with immediate access to the products of the N fixation. The mistletoe also acquired some fixed N from the xylem sap of the acacia (Ku and Pate 1997; Tennakoon and Pate 1997). Similarly, the root hemiparasites, *Santalum* spp., acquired N from the xylem sap of *A. ampliceps* and *A. trachycarpa* hosts (Tennakoon et al. 1997a; Radomiljac et al. 1998).

Two insect species, *Sitona* sp. and *Rivellia* sp., are known to attack legume nodules (Diatloff 1965; Gibson 1977), and might thus affect N fixation. Diatloff (1965) reported that *Rivellia* larvae damaged 50–70% of nodules on perennial glycine — *Glycine* (now *Neonotonia*) *wightii*, but there is no record of these insects infesting acacia nodules.

Like other plants, acacias are subject to attack by insects — see e.g. Searle (2000) — and fungal pathogens — see e.g. Old (1998). It is not known what effects such attacks have on symbiotic N fixation. However, it is a rule of thumb with legumes that any factor that reduces plant growth also lessens N fixation (cf. Peoples et al. 1998; Peoples and Baldock 2001).

Acacias themselves may attain pest status — see e.g. Holmes and Cowling (1997). *Acacia dealbata*, an Australian native species, is a serious weed in Chile, India and South Africa due to its prolific seed production and suckering habit (Doran and Turnbull 1997). Stock et al. (1995) reported that the N fixation of alien species *A. cyclops* and *A. saligna* was a significant factor in their invasion of two South African ecosystems and their establishment, persistence and successful competition with

indigenous flora. *Acacia smallii* (now *A. farnesiana* var. *farnesiana*) was recorded as a serious invader of grassland (Polley et al. 1997); it is not known if its N-fixing ability was a factor. In Australia, native acacias such as *A. baileyana* and *A. longifolia* sometimes become weeds.



*Acacia baileyana* (Cootamundra wattle) makes an attractive ornamental for parks and gardens. Unfortunately, it may become an invasive weed of bushland outside its natural environment.

### MYCORRHIZAL FACTORS AFFECTING NITROGEN FIXATION IN THE FIELD

Many plant species benefit from associations with mycorrhiza, mainly because of the ability of the fungi to act as conduits for plant nutrients, scavenged from infertile soil, that would be otherwise inaccessible to the plant. The associations tend to be of greatest benefit to the plant under conditions of low fertility. Indeed, even moderate amounts of fertiliser — e.g. phosphate (Kahiluoto et al. 2000) — depress growth of mycorrhiza and

reduce the infectivity and efficiency of the fungi. In agroforestry, mycorrhizal associations contribute much to the growth of *Acacia* species in unfertilised fields (Dart et al. 1991). Reddell and Warren (1987) listed nearly 50 species of *Acacia* with mycorrhizal associations. Some aspects of the management of mycorrhizas in forestry have been dealt with by Grove and Malajczuk (1994) and Jasper (1994). Reddell and Warren (1987) drew attention to the potential for using inoculants of mycorrhizal fungi to improve the survival, establishment and growth of tropical acacia plantations. They speculated that nursery inoculation of seedling stock destined for outplanting into the field might be an efficient means of fulfilling this potential.

Acacias form associations with both endomycorrhiza and ectomycorrhiza (Reddell and Warren 1987). Endomycorrhiza — commonly known as vesicular-arbuscular mycorrhiza (VAM) — invade the roots. Ectomycorrhiza colonise the root surfaces. Acacias respond to inoculation with either type (e.g. Dela Cruz and Yantasath 1993; Osunubi et al. 1996; Munro et al. 1999). Some Australian acacias associate with both types of mycorrhiza and form root nodules (Sprent 1994b) as well. While endomycorrhiza occur frequently in soils growing acacias, ectomycorrhiza are less common and may be absent from some soils (Khasa et al. 1994). Combined mycorrhiza and root-nodule bacteria (cf. Mosse et al. 1976) may synergistically stimulate N fixation in legumes growing in soil that is deficient in plant-available P; for example, Dela Cruz and Yantasath (1993) noted enhanced growth of *A. mangium* following inoculation with both mycorrhiza and rhizobia. Beniwal et al.

(1992) and Mandal et al. (1995) demonstrated growth responses in *A. nilotica* to co-inoculation with rhizobia and mycorrhizal fungi, but did not examine the effect of each organism individually. Lal and Khanna (1996) noted that the growth of *A. nilotica* after joint inoculation with one rhizobial strain and *Glomus fasciculatum* was better than after inoculation with either organism individually. This synergism appeared to be a specific effect involving that particular strain of rhizobium rather than a general phenomenon. Ba et al. (1994) observed that the ectomycorrhiza, *Pisolithus tinctorius*, interfered with rhizobial infection thread development and nodule meristem initiation in *A. holosericea*. These results were obtained in axenic culture in the laboratory. It seems unlikely that they would extrapolate to the complex microfloral conditions of the field.

Using the natural abundance technique, Michelsen and Sprent (1994) found that some vesicular-arbuscular mycorrhiza improved N fixation by four *Acacia* species growing in a nursery, although there was no corresponding increase in shoot N concentration. Franco et al. (2001) considered that joint inoculation of tree legumes with rhizobia and mycorrhiza held promise as an aid to land reclamation in the humid Amazon. Chung et al. (1995), on the other hand, found no benefit from co-inoculation. This work was done with hybrid plantlets from tissue culture. *Acacia confusa* and *A. mangium* in pot experiments responded to dual inoculation with vesicular-arbuscular mycorrhizal fungi and phosphorus-solubilising bacteria (Young 1990); the *Acacia* species may not have been nodulated. Ba et al. (1996) considered



that the use of ectomycorrhiza can contribute to an increase in the N-fixing potential of *A. holosericea* and *A. mangium* and were optimistic that endomycorrhiza might have the same value. These views mirror our own conclusions about the association between mycorrhiza and N fixation by acacias generally. In some instances, the tripartite relationship including rhizobia can be synergistic but the conditions required for that to occur are not well defined.

A more subtle influence on the N economy of acacias appears to lie in the ability of mycorrhiza to access multiple forms of N from the soil. Whereas plants, including trees (Devisser and Keltjens 1993; Turnbull et al. 1995) such as acacias, are largely restricted to the use of nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) from the soil and, for legumes, N from the atmosphere, mycorrhiza is more acquisitive. There is now abundant evidence, cited by Boddey et al. (2000b), that mycorrhizal fungi and the plants that they infect are able to absorb from the soil, in addition to  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , amino acids and N from proteins and chitin. Dommergues (1982) was sufficiently impressed with the potential benefits of mycorrhizal infection to N-fixing trees to suggest that, as well as inoculation with rhizobia, ectomycorrhizal and endomycorrhizal inoculants should be considered. It is of interest to note that Cantrell and Linderman (2001) observed that pre-inoculation of lettuce (*Lactuca sativa*) and onion (*Allium cepa*) with endomycorrhizal fungi reduced harmful effects of soil salinity. It is not known whether mycorrhizas confer similar benefits on acacias growing in saline environments.

## **RHIZOBIAL FACTORS INFLUENCING NITROGEN FIXATION IN THE FIELD**

### **General considerations**

There are always three aspects of the legume symbiosis to consider when appraising factors that affect the efficiency of N fixation: the plant, the bacteria and the interaction between them. Variation can and does occur in each of these aspects. It is particularly significant in the host plant and in the rhizobia, and has major implications for the establishment of a symbiosis that is effective in fixing N.

### **Genetic variability of host acacias**

During an examination of more than 40 lines of *A. nilotica*, Beniwal et al. (1995) and Toky et al. (1995) recorded marked genetic variation among provenances in ability to fix N. A similar study by Burdon et al. (1998) of 67 populations of 22 *Acacia* species and associated strains of rhizobia from south-eastern Australia found little evidence of rhizobia strain/*Acacia* provenance effects on N fixation. They concluded that elite rhizobial cultures from one provenance would perform well on other provenances. However, significant host-based variation in the capacity to form effective symbiotic associations was detected in half-sib families of *A. dealbata*, *A. mearnsii* and *A. melanoxylon*. This led to the suggestion that, in acacia breeding programs, it would be prudent to continually monitor the N-fixing capacity of breeding material. Similarly, Sun et al. (1992c) used rhizobial inoculation and application of combined N to study the symbioses of multiple provenances

of *A. auriculiformis*, *A. mangium* and *A. melanoxylon*. Significant variation in nodulation and responses to applied N led them to conclude that there was scope to increase growth by plant selection. Sprent (1995) also considered that there was substantial potential to enhance acacia symbiosis by plant selection. Zorin et al. (1976) demonstrated with Caucasian clover (*Trifolium ambiguum*), growing axenically in tubes of vermiculite moistened with McKnight's nutrient solution (McKnight 1949), that a meaningful preliminary selection for increased N fixation could be achieved within 28 days. Like *T. ambiguum*, species of *Acacia* are poorly domesticated, vary in symbiotic response from plant to plant (Burdon et al. 1998, 1999) and can be grown under bacteriological control (Kang et al. 1998). Therefore, the procedure might feasibly be used among acacias and their rhizobia for selecting for enhanced symbiosis.

### Cross nodulation

The concept of cross nodulation, a manifestation of host/rhizobial specificity, also known as cross inoculation, was developed in early studies of the symbiosis between legumes and root-nodule bacteria (Fred et al. 1932). The concept dictates that related groups of legumes are nodulated by particular rhizobia, and that those rhizobia will nodulate across all legumes in the group (cross-nodulation group) but will not nodulate plants in other cross-nodulation groups. The classical version of the system of cross-nodulation groupings is portrayed in Table 7. There are clear anomalies in the system: (i) the groupings are selective, with six groups accounting for about 1000 species and the

other group, the so-called cowpea miscellany, for about 19,000 species across all legume families, Mimosaceae, Caesalpiniaceae and Fabaceae; (ii) single tribes (Trifolieae, Phaseoleae) are represented in more than one cross-nodulation group; (iii) genera in the same cross-nodulation group (*Lupinus*, *Ornithopus*) belong to different tribes; (iv) the cowpea group is clearly 'miscellaneous', extremely diverse and merely a 'catch-all'. By implication, *Acacia* was allocated to the cowpea group. This was accurate in that rhizobia isolated from *Acacia* spp. formed nodules, and often fixed N, with *Vigna unguiculata* (cowpea).



Alison Jeavons

Many strains of rhizobia that nodulate *Acacia* spp. are often highly promiscuous in that they also form nodules, and may fix nitrogen, with many other species including those from different families. This phenomenon is an example of 'cross-nodulation'. The Australian native shrub, *Daviesia ulicifolia* (gorse bitter pea) — family Fabaceae, cross-nodulates with *Acacia* spp. — family Mimosaceae.

Despite all its anomalies, the concept of cross nodulation had practical value. It provided a convenient guide for manufacturers of commercial legume inoculants used in agriculture because it allowed them to use a single strain of rhizobia, and therefore a single inoculant, for a number of different legumes of the same cross-nodulation group. In Australia, for instance, *Rhizobium*

*leguminosarum* bv. *trifolii* strain TA1 was, and still is, the sole rhizobial component of commercial inoculant for seven species of *Trifolium*: *T. alexandrinum* (berseem clover), *T. dubium* (suckling clover), *T. fragiferum* (strawberry clover), *T. glomeratum* (cluster clover), *T. hybridum* (alsike clover), *T. pratense* (red clover) and *T. repens* (white clover) (Brockwell et al. 1998).

**Table 7.** Portrayal of an early version of the system of legume cross inoculation (cross nodulation) grouping

Group	Genus	No. of species <sup>a</sup>	Tribe	Family	Root-nodule bacterium <sup>b</sup>
Clover	<i>Trifolium</i>	250–300	Trifolieae	Fabaceae	<i>Rhizobium leguminosarum</i> bv. <i>trifolii</i>
Medic/ melilot	<i>Medicago</i>	50–100	Trifolieae	Fabaceae	<i>Sinorhizobium meliloti</i>
	<i>Melilotus</i>	20	Trifolieae	Fabaceae	<i>S. meliloti</i>
	<i>Trigonella</i>	70–75	Trifolieae	Fabaceae	<i>S. meliloti</i>
Pea	<i>Pisum</i>	6	Viciae	Fabaceae	<i>R. leguminosarum</i> bv. <i>viciae</i>
	<i>Lathyrus</i>	130	Viciae	Fabaceae	<i>R. leguminosarum</i> bv. <i>viciae</i>
	<i>Lens</i>	5	Viciae	Fabaceae	<i>R. leguminosarum</i> bv. <i>viciae</i>
	<i>Vicia</i>	150	Viciae	Fabaceae	<i>R. leguminosarum</i> bv. <i>viciae</i>
	( <i>Vavilovia</i> )	(1)	Viciae	Fabaceae	<i>R. leguminosarum</i> bv. <i>viciae</i>
Bean	<i>Phaseolus</i>	50–100	Phaseoleae	Fabaceae	<i>R. leguminosarum</i> bv. <i>phaseoli</i>
					<i>R. etli</i>
					<i>R. tropici</i>
Lupin	<i>Lupinus</i>	150	Genisteeae	Fabaceae	<i>Bradyrhizobium</i> sp. ( <i>Lupinus</i> )
	<i>Ornithopus</i>	15	Hedysareae	Fabaceae	<i>Bradyrhizobium</i> sp. ( <i>Lupinus</i> )
Soybean	<i>Glycine</i> <sup>c</sup>	2 <sup>c</sup>	Phaseoleae	Fabaceae	<i>B. japonicum</i>
Cowpea <sup>d</sup>	All others	ca 19,000	Very many tribes	Fabaceae	Many species; fast- and slow-growers
				Mimosaceae	Many species; fast- and slow-growers
				Caesalpiniaaceae	Several species; mainly slow-growers

Source: derived from Fred et al. (1932).

<sup>a</sup> According to Allen and Allen (1981).

<sup>b</sup> According to Young (1996).

<sup>c</sup> *Glycine max* and *G. soja* only; other species of the genus *Soja* allocated to the cowpea group.

<sup>d</sup> Species of the genus *Acacia* allocated to the cowpea group.

During the past decade, the concept of cross nodulation has been modified by advances in rhizobial taxonomy (e.g. Young 1996; Young and Haukka 1996). A consequence is that rhizobial nomenclature is no longer determined by the host range of the organism, but by molecular techniques. The host range of the organism is then determined quite independently. Despite this change of emphasis, the modern version of phylogenetic relationships in the Rhizobiaceae (Table 3) still has substantial relevance to cross nodulation and retains implications for the selection of strains for use in legume inoculants. Table 3 shows that (fast-growing) isolates of rhizobia from *Acacia* belong to *Sinorhizobium saheli*. Some symbiotic affinity between *Acacia* rhizobia (*Sinorhizobium saheli*) and species of *Leucaena*, which is normally nodulated by strains of the genus *Rhizobium*, has been demonstrated by Swelin et al. (1997). There are many similar anomalies. Undoubtedly, other (slow-growing) acacia rhizobia are unnamed species of *Bradyrhizobium*. Very-slow-growing isolates may have affinities to *Bradyrhizobium liaoningense*.

The term 'cross nodulation' simply implies that a rhizobial strain has the ability to *form nodules* on the legumes in its cross-nodulation group. It does not necessarily mean that those nodules will *fix nitrogen*. The genus *Trifolium* provides examples of this constraint. In general, clovers of the Mediterranean region fix N with one particular set of strains of *R. leguminosarum* bv. *trifolii*, whereas clovers of central African origin require a distinctly different set of strains for N fixation. Clovers from the Caucasus Mountains (north-eastern Turkey, southern Russia and Georgia) form a third cluster of rhizobial

requirement for N fixation, clovers from the Rocky Mountains in North America a fourth, South American clovers a fifth, and so on (Brockwell 1998). It seems likely that these different requirements for effective strains of *R. leguminosarum* bv. *trifolii* are a consequence of the different clusters of *Trifolium* species having evolved in isolation from one another. It represents a fine example of host/rhizobial specificity. In contrast, acacias from Australia, Africa and Asia are, more often than not, nodulated by each other's rhizobia. This is an example of host/rhizobial promiscuity.

The concepts of specificity and promiscuity have important practical implications for the manipulation of the symbiosis between legumes and rhizobia in order to enhance nodulation and improve N fixation. It has been known for more than a century that not all legumes are nodulated by all rhizobia. The extent to which a rhizobial strain can infect and form nodules on different legumes is an inverse measure of its specificity. The more legumes it can nodulate the less specific, or more promiscuous, it is. A strain that has a very narrow host range is considered to be highly specific. Identical terminology is used for the host plant. A legume that accepts infections from and nodulates with only a small number of rhizobial strains is termed specific; one that nodulates with many is promiscuous. The concept of specificity applies to N fixation as well as to nodulation. Combinations of plant and bacterium that form nodules do not always fix N. A consequence of this is that legumes and rhizobial strains are, as a rule, more specific for N fixation than for nodulation.





On the basis of ability to form nodules, many *Acacia* species can be classed as promiscuous whereas, based on ability to fix N, some of those same species are clearly specific. An example is *A. caven* (Frioni et al. 1998a,b). Working with a collection of strains of *Bradyrhizobium*, Turk and Keyser (1992) concluded that *A. mangium* was promiscuous for nodulation but specific for N fixation, whereas *A. auriculiformis* was promiscuous for both nodulation and N fixation. Woldemeskel and Sinclair (1998) went further and identified rhizobial strain specificity at subspecies and provenance levels in *A. nilotica*. Burdon et al. (1999) went further still and drew attention to the occurrence of specificity in N fixation between individual acacia seedlings. In Senegal, different provenances of *A. albida*, approximately equal in total dry matter and N content, differed widely (0–38% Ndfa) in the proportion of plant N due to N fixation (Gueye et al. 1997). The ability of *A. albida* to scavenge N from

the soil to compensate for symbiotic inefficiency is a trait that it apparently shares with other legumes, e.g. *Medicago polymorpha* (common burr medic) (Bowman et al. 1998).

We note that cross-nodulation occurs right across the three families of legumes. This implies that the root-nodule bacteria (rhizobia) from plants of one family have the capacity to form nodules and, perhaps, to fix N with some members of each of the other two families. It is likely, nevertheless, that the symbiotic associations between some acacias and their rhizobia are quite specific, with little or no cross-nodulation with other species. On the other hand, rhizobia isolated from acacia nodules may have the ability to nodulate distantly related legumes. For instance, Swelin et al. (1997) noted the sparse nodulation of leucaena (*Leucaena leucocephala*) by strains of *Bradyrhizobium* sp. (*Acacia*).



Alison Jeavons

*Kennedia prostrata* (running postman) — family Fabaceae — cross-nodulates with *Acacia* spp. An Australian native, it is sometimes used as an ornamental ground cover.



Alison Jeavons

The creeping shrub, *Hardenbergia violacea* (false sarsaparilla) — family Fabaceae — is another Australian native plant that cross-nodulates with *Acacia* spp. *Hardenbergia violacea* is a popular ornamental.

Lorquin et al. (1997) determined the structures of *nod* factors produced by strains of *Sinorhizobium teranga* bv. *acaciae* and *Rhizobium loti* U cluster — almost certainly synonymous with what is now known as *Mesorhizobium plurifarum* (de Lajudie et al. 1998b) — both of which nodulate *Acacia* species. Compounds from the two organisms were similar, indicating a close relationship between *nod*-factor structure and host specificity, independent of the taxonomic classification of the rhizobia.

### Rhizobial specificity and promiscuity

As might be expected in so large and diverse a genus, there are all degrees of host/rhizobial specificity in the *Acacia* symbiosis, varying from highly specific to widely promiscuous (e.g. Habish and Khairi 1970; Roughley 1986; Bowen et al. 1999). Indeed, some extraordinarily promiscuous rhizobia, e.g. *Rhizobium* sp. strain NGR234 isolated from the non-legume *Parasponia andersonii*, have a diverse host range of 300–400 species including acacias (Pueppke and Broughton 1999). The literature holds a number of examples of specificity and promiscuity relating to both nodulation and N fixation by acacias. Njiti and Galiana (1996) classified the tropical, dry-zone species, *A. albida*, *A. holosericea*, *A. nilotica*, *A. polyacantha* and *A. senegal*, as promiscuous in that they all nodulated and fixed N indiscriminately with strains of both *Rhizobium* and *Bradyrhizobium*. In contrast, Dommergues (1982) demonstrated more specific reactions, as interactions between two groups of acacia hosts and diverse rhizobia. One group fixed N with fast-growing strains (*Rhizobium*, and probably *Sinorhizobium* and *Mesorhizobium*) but not with slow growers (*Bradyrhizobium*); the

other group exhibited reverse reactions. Ndiaye and Ganry (1997) recognised a consequence of promiscuity. When they detected low levels of N fixation in *A. albida* growing in the field in Senegal, they attributed it to paucity or absence of appropriate rhizobia in the soil. Trinick (1980) located fast-growing strains that cross-nodulated with *A. farnesiana* and species of *Lablab*, *Leucaena*, *Mimosa* and *Sesbania*. Sanginga et al. (1989a) reported that some slow-growing strains — of *Bradyrhizobium* sp. (*Faidherbia*) — isolated from *A. albida* could form nodules on leucaena (*Leucaena leucocephala*). Rasanen et al. (2001) noted that strains of four species of *Sinorhizobium* isolated in Sudan and Senegal nodulated and fixed N with eight *Acacia* species and four *Prosopis* species of African or Latin American origin, but not with *Sesbania rostrata*. Lopez-Lara et al. (1993, 1995) noted that a strain of *Rhizobium* sp. from *A. cyanophylla* had a very wide host range that included *Trifolium* species. They implicated the composition of surface polysaccharides in the promiscuity. Wang and Wang (1994), on the other hand, reported that a strain of rhizobia similar to *Sinorhizobium meliloti*, which had been isolated from *A. auriculiformis*, nodulated its host and *A. confusa* at high frequency, *A. mangium* at low frequency, but formed no nodules at all on *A. mearnsii*. Only one of 12 strains of acacia rhizobia isolated from *A. mangium* in the Philippines formed effective nodules on its host (Dart et al. 1991).

Thompson et al. (1984) took 38 strains of rhizobia isolated from Australian native legumes, including acacias, and used them in a glasshouse experiment to inoculate 63 species of *Acacia*. The majority of species (45/63) formed nodules with more than

75% of the rhizobial strains, 11/63 nodulated with between 50% and 75% of strains and 7/63 with less than 50%. No data were presented for N fixation. Dart et al. (1991) demonstrated a wide range of specificity and symbiotic effectiveness when they tested 48 strains of acacia rhizobia of diverse origin on *A. auriculiformis* and *A. mangium*. *Acacia auriculiformis* was very much more promiscuous in terms of N fixation than *A. mangium*. They used these and related findings as a basis for selecting N-fixing rhizobia for particular acacia species (Bowen et al. 1999). Burdon et al. (1999) describe a remarkable instance of *Acacia* host/*Acacia* rhizobia specificity in *A. dealbata*. Ten half-sib families were each inoculated with a single strain of rhizobia. In nine of the families, N fixation was ineffective, whereas the tenth fixed N vigorously.

### **Variation in the symbiotic reaction between host and rhizobia**

The degree of specificity shown by a species of *Acacia* or a strain of rhizobium is undoubtedly a function of the particular combination of plant and bacterium used to make the determination. Table 8 shows an example taken from the work of Turk (1991), who tested three *Acacia* species against a diverse collection of strains from three genera of rhizobia. The responses in terms of nodule formation are complex, but it is clear from the data that the complexities of the interactions would be different had a smaller subset of the strains, or another collection of strains, been used for making the determination.

Souvannavong and Galiana (1991) presented an example of how the interaction between legume

and micro-organism might be exploited to enhance the symbiosis. They found evidence that N fixation might be increased by selecting both the lines of *A. mangium* and the rhizobia that nodulate it.

### **Appraisal of specificity as a constraint to effective field nodulation of acacias**

While it is clear that *Acacia* host/*Acacia* rhizobia symbioses are extremely variable, we conclude that most associations between the symbionts in the wild usually fix some atmospheric N. Although the amount fixed varies considerably, we believe that the symbiosis itself is unlikely to be the major determinant of how much N is actually fixed. It seems to us that it is much more likely that environmental factors, particularly the presence of soil N as  $\text{NO}_3^-$ , but also soil reaction, soil moisture, soil salinity, nutrient deficiency, insufficient light, and the influence of pests, limit the amount of N fixed by naturally occurring acacias. It is only when limiting factors such as these are not operating that the N-fixing ability of a legume/rhizobium association will be capable of expressing its full potential. Then, and only then, might partial effectiveness of the symbiosis be a factor limiting N fixation. These arguments would not, of course, apply to those cases when the symbiotic association between plant and bacteria is ineffective. However, we suspect that the occurrence of truly ineffective associations in the field is very rare. An appraisal of the literature that we have cited supports this view.

A form of selective preference for particular bacteria for nodule formation has been recorded for *Acacia* species by Odee et al. (1998). They found

that, when grown in various African soils, each containing a diverse rhizobial microflora, *A. albida*, *A. auriculiformis* and *A. holosericea* nodulated largely with *Bradyrhizobium* and only occasionally with *Rhizobium*, whereas *A. polyacantha* and *A. tortilis* grown in the same soils nodulated exclusively with *Rhizobium*. Certain legumes (e.g. *Trifolium* spp.) exercise a more specific form of selective preference by selecting for their nodulation the more effective strains from a mixed rhizobial microflora — see e.g. Robinson (1969) and Masterson and Sherwood (1974). There is some unconfirmed evidence that this phenomenon might also occur in *Acacia* (Table 9).

Nevertheless, even in circumstances where the N-fixing capacity of field populations of acacia rhizobia is deficient, it would seem ecologically and economically impossible to introduce new, more effective strains into the soils of established forests. Enhancement of the amount of N fixed by forest acacias might be feasible by correction of soil nutrient deficiencies but even that is probably impracticable. Notwithstanding, we believe that there is substantial scope for exploiting the symbiosis to improve the health and vigour of acacias newly established in plantations, for farm forestry or in land rehabilitation (e.g. Thrall et al. 2001a,b).

**Table 8.** Nodulation of three species of *Acacia* inoculated with 34 diverse strains representing three genera of root-nodule bacteria

Species	No. of strains	<i>A. auriculiformis</i>	<i>A. mangium</i>	<i>A. mearnsii</i>
<i>Rhizobium</i>	18			
	2	Plus <sup>a</sup>	Plus	Plus
	2	Plus	Plus	Minus <sup>a</sup>
	2	Plus	Minus	Minus
	3	Minus	Plus	Plus
	2	Minus	Plus	Minus
	7 <sup>b</sup>	Minus	Minus	Minus
<i>Bradyrhizobium</i>	15			
	6 <sup>c</sup>	Plus	Plus	Plus
	1	Plus	Plus	Minus
	3	Plus	Minus	Plus
	1	Minus	Plus	Plus
	1	Minus	Plus	Minus
	2	Minus	Minus	Plus
	1	Minus	Minus	Minus
<i>Azorhizobium</i>	1			
	1	Plus	Plus	Plus

Source: data extracted from Turk (1991).

<sup>a</sup> Plus = nodules; Minus = no nodules.

<sup>b</sup> Three strains from *Robinia*, not classified by Turk (1991), thought to be *Rhizobium*.

<sup>c</sup> One reaction, not determined, thought to be positive (Plus).

**Table 9.** Evidence for selective preference\* by *Acacia* species for the more effective rhizobial strain components of a mixed-strain inocula

<b>(a) Effectiveness index for three strains of acacia rhizobia in association with three <i>Acacia</i> species. (Index is the whole plant dry matter increment due to inoculation)</b>			
<b>Strain of rhizobia</b>	<b><i>A. implexa</i></b>	<b><i>A. melanoxylon</i></b>	<b><i>A. mearnsii</i></b>
53A-21	0.90a	1.25ab <sup>†</sup>	1.18a
49A-20	0.84a	1.55a	1.26a
4207	0.71a	0.79b	0.94a
<b>(b) Responses (dry matter — mg/plant) of three <i>Acacia</i> species to inoculation with three single-strain inocula and a multi-strain inoculum</b>			
<b>Strain of rhizobia</b>	<b><i>A. implexa</i></b>	<b><i>A. melanoxylon</i></b>	<b><i>A. mearnsii</i></b>
		<i>Single-strain inocula</i>	
53A-21	415ab <sup>†</sup>	301ab	358a
49A-20	384ab	351a	367a
4207	367b	238c	312a
		<i>Multi-strain inoculum</i>	
All three	443a	341ab	325a

\* Selective preference is indicated when the response to inoculation with a mixed-strain inoculum (of 2 or 3 strains) is equal to or greater than the response to inoculation with any one of the strains as a single-strain inoculum. For instance, strain 4207 has a relatively poor effectiveness index (see part a) with each of the three *Acacia* species. When 4207 is used as a single-strain inoculum, its N-fixing performance (with each of the three species) is inferior to the performance of multi-strain inocula of which 4207 is a component (see part b); i.e. the *Acacia* species have exercised a selective preference for the better strains in the mixed-strain inocula.

<sup>†</sup> Values in each row with a common letter are not significantly different from one another ( $P > 0.05$ ).

## 5. Enhancement of the symbiosis

### THE NEED FOR INOCULATION

*Acacia* rhizobia are, as we have mentioned, as widely distributed as the *Acacia* species themselves. Indeed, they sometimes occur spontaneously in anthropogenic environments, e.g. landfill in Hong Kong (Chan et al. 1998). Nonetheless, there are many soils where the population density is so low as to pose a threat to the establishment of N-fixing trees (Thrall et al. 2001a). There will be other soils where suitable strains for rhizobia-specific species will be absent. Used judiciously according to need, and performed properly, legume inoculation is a significant agency for improving plant productivity and soil fertility. 'Is it necessary to inoculate?' is a question that has been approached in different ways (Table 10).

Field experiments designed to diagnose the need for inoculation (e.g. Brockwell 1971; Date 1977; Thies et al. 1991a) are unsuitable for acacias. Bonish (1979) and Brockwell et al. (1988) used dilutions of soil samples to inoculate clover seedlings growing under aseptic conditions in test tubes to demonstrate a quick, microbiological means for characterising simultaneously the size and N-fixing capacity of soil-borne populations of rhizobia. The method could, no doubt, be refined for use as a diagnostic of the need for inoculation of acacias. A related procedure (Thies et al. 1991b) makes it possible to forecast

the likely success of introducing inoculant rhizobia into the soil by considering indices of the size of the resident rhizobial population and the N status of the soil (cf. Singleton and Tavares 1986). Thies et al. (1994) put forward a unique proposal for predicting the need for inoculation on a regional basis using a geographical information system.

As our literature survey has shown, there are, among acacias, all degrees of host/rhizobial specificity in the symbiosis, varying from widely promiscuous to highly specific. Species in the latter category are those most likely to need and benefit from inoculation upon introduction into new environments where soils lack specific N-fixing rhizobia.

### RESPONSES TO RHIZOBIAL INOCULATION OF ACACIAS IN FIELD AND NURSERY

Masutha et al. (1997) suggested that promiscuous species of *Acacia*, capable of nodulation and N fixation with naturalised populations of rhizobia, should be chosen for use in agroforestry programs, implying that they considered natural inoculation of acacias by resident soil rhizobia to be superior to artificial inoculation with cultures of rhizobia. We accept that proposition in situations where the numbers of acacia rhizobia in the soil are so large

(say >1000 cells per gram) that introduced strains would encounter extreme competition from the resident organisms. Where, however, naturalised populations are smaller, there are excellent prospects of successfully introducing inoculant strains, provided sensible strategies are employed.

While there are only a few field reports of acacias responding to rhizobial inoculation, they document careful work and convincing results. Working with *A. mangium* in Ivory Coast, Galiana et al. (1994) reported that inoculation had a positive effect on tree growth for more than three years after outplanting. Moreover, one of the inocula persisted well and could be re-isolated from root nodules up to

42 months after transfer of the inoculated trees to the field. This particular organism (Aust13c) appears to have considerable potential as a strain for making inoculants. In Ivory Coast, 50–90% of total nitrogen in *A. mangium* trees was attributed to fixation of atmospheric N (Galiana et al. 1996). Galiana et al. (1998) reviewed these works and similarly successful experiments with *A. mangium* that had been conducted in other tropical countries. Lal and Khanna (1996) demonstrated a field response to inoculation of *A. nilotica* grown in India.

The long-term success of inoculation in the field appears to depend on the initial establishment of a vigorous crop of nodules. The nodule itself

**Table 10.** Indicators of the need to inoculate legume seed with effective rhizobia at time of sowing

**Allen and Allen (1961) — historical indicators**

1. The absence of the same or of a symbiotically related legume in the immediate past history of the land
2. Poor nodulation when the same species was grown on the land previously
3. When a legume follows a non-legume in a rotation
4. In land reclamation undertakings.

**Roughley and Brockwell (1987) — microbiological queries**

1. How specific is the legume in its rhizobial requirements?
2. What is the likelihood of effective rhizobia spreading from volunteer legumes?
3. Has the legume been sown previously and for how long was it grown continuously?
4. How long since the legume was last grown and, in the meantime, did conditions favour persistence of the rhizobia?

**Thies et al. (1991b) — queries relating to soil indices**

1. How large is the resident population of competitive rhizobia?
2. What is the level of soil nitrogen?

Source: after Brockwell et al. (1995a).

represents an environment akin to pure culture and, within it, there is great multiplication of the rhizobia. When, subsequently, nodule breakdown takes place, large numbers of viable cells are released into the soil (e.g. Reyes and Schmidt 1979; Kuykendall et al. 1982; Moawad et al. 1984; Thies et al. 1995) where they constitute a potent source of infection for new roots and may become a permanent component of the soil microflora even in the presence of competing organisms.

The total area of land worldwide under acacia plantations exceeds 5 million hectares. It is probable that most of these trees were outplanted as nursery

tube stock. This practice can be exploited for inoculation. Acacias can be inoculated at the time of sowing in the nursery in a way that ensures that the seedling trees are vigorous, well-nodulated and fixing N at the time of outplanting into the field. This has been demonstrated at nurseries in Australia (Brockwell et al. 1999a). When nursery-inoculated seedlings were outplanted, their survival, growth and benefit to companion plantings of *Eucalyptus nitens* (shining gum) was better than that of uninoculated seedlings (Table 11).

Wherever restoration of native vegetation and re-establishment of tree cover over large areas

**Table 11.** Effect of inoculation by soil enrichment on growth of *Acacia mearnsii* in nursery and field. (N fixed calculated using natural abundance of  $^{15}\text{N}$  with *Eucalyptus nitens* as the non-N-fixing reference plant)

	<i>A. mearnsii</i>		LSD ( $P=0.05$ )
	Inoculated	Uninoculated	(n.s. — $P>0.05$ )
Nursery			
Seedlings nodulated (%) — day 126	100.0	35.0	33.0
Seedlings nodulated (%) — day 231	100.0	70.0	n.s.
Nodule score (0-5) — day 126	4.00	0.60	0.82
Nodule score (0-5) — day 231	3.00	0.95	0.73
Shoot DM (g/plant) — day 231	1.78	1.27	n.s.
Shoot DM (mg/plant) — day 231	35.7	26.4	14.6
Shoot N fixed (%) — day 126	68.3	44.4	5.9
Shoot N fixed (%) — day 231	88.3	25.3	11.8
Shoot N fixed <sup>a</sup> (mg/plant) — day 126	31.6	7.8	12.3
Field <sup>a</sup>			
Survival (%) — day 231 to day 280	96.3	90.7	4.6
Survival (%) — day 280 to day 553	98.1	91.4	3.4
Tree height (cm) — day 280	28.3	21.2	1.2
Tree height (m) — day 553	2.03	1.82	0.9

Source: after Brockwell et al. (1999a).

<sup>a</sup> Seedling trees outplanted into the field after 231 days in the nursery.



are major conservation issues, as in some parts of Australia, the use of tube stock becomes impracticable. Where acacias (or other leguminous trees or shrubs) are appropriate for such circumstances, it may be a more practical sowing strategy to use direct drilling or aerial seeding (Thrall et al. 2001a,b). As part of the methodology, it would be necessary to incorporate rhizobial inoculant in a seed pellet (e.g. Brockwell 1962) or to deliver the inoculant in granular form (e.g. Scudder 1975) into the seed furrow close to the seed. It is suggested that direct drilling or aerial seeding approaches using multi-strain inocula (see below) incorporated in seed pellets should be adopted as a general strategy for revegetation of degraded landscapes with shrubby legumes in Australia and, perhaps, other parts of the world where native forest has been removed. Used in conjunction with the replanting of *Eucalyptus* and/or other non-leguminous species, the benefits are likely to be enhanced. Overall, the development of practical approaches and uncomplicated techniques suitable for large-scale, low-cost establishment of native legumes is likely to lead to more efficient revegetation and soil management by landholders, and greater incentive to invest in reclamation projects due to affordable costs and higher survival rates of the sown species.

The paucity of literature relating to field inoculation of acacias indicates that most plantings rely on spontaneous nodulation from naturalised populations of rhizobia. Clearly, acacia symbioses with effective rhizobia are not properly exploited. Indeed, at the time of writing, we are unaware of any inoculant manufacturer anywhere in the world

who produces (except by special request) rhizobial cultures for *Acacia* species. Until that situation is rectified, this important natural, renewable resource will remain under-exploited. Inoculant preparation involves only a few, simple, well-established steps.

## INOCULANTS

The principles of inoculant production are well documented — e.g. Burton (1982); Thompson (1983); Somasegaran and Hoben (1994); other work cited by Brockwell et al. (1995a). The selection of carrier material is a critical determinant of inoculant quality. Finely ground peat (Thompson 1980) has been the inoculant carrier of popular choice for many years, though there are other promising carriers (Brockwell and Bottomley 1995). Roughley and Vincent (1967) and Date and Roughley (1977) record that inoculants prepared with sterile peat contain 100-fold more rhizobia than those made with non-sterile peat. Moreover, because mortality of rhizobia is greater in unsterilised peat, the difference increases during storage.

### Strain selection

Some principles of matching acacia species and acacia rhizobia have been catalogued by Brockwell (1998). A list of characters considered desirable for rhizobial strains for legume inoculants is shown in Table 12. It is obviously not feasible to test strains for all of these characters. Naturally, the ability to form nodules and fix N on the target legume are the essential characteristics. Methods for testing for strain effectiveness for legumes in general have been described by, amongst others, Vincent

(1970), Gibson (1980) and Somasegaran and Hoben (1994). Methods adapted or specifically designed for acacias have been used by Thompson et al. (1984), Umali-Garcia et al. (1988), Galiana et al. (1990) and Burdon et al. (1999). Using such methods, Brockwell et al. (1999b) and Dippel et al. (1999) made recommendations for inoculant strains for a range of acacias to be raised in plant nurseries and destined for plantation and farm forestry. Martin-Laurent et al. (1997) demonstrated that aeroponic culture led to abundant nodulation of inoculated *A. mangium*. The method lends itself to studies of infection processes and nodule morphology and, if it can be adapted, to selection of effective rhizobial strains.

As noted earlier, there are all degrees of host/rhizobial specificity in the acacia symbiosis. However, a pragmatic approach to strain selection is essential for production of acacia inoculants because it would not be feasible to have a special inoculant for every different acacia. With respect to the N-fixing potential of any particular acacia/inoculant strain combination, we advocate the principle of seeking the best result possible rather than the best possible result. The findings of Burdon et al. (1999) and Thrall et al. (2000) give some encouragement. In studies of symbiotic associations between temperate Australian acacias and populations of native rhizobia, they found a general lack of host/rhizobia interaction effects (with notable exceptions) and concluded that, where no rhizobial strain for a particular host species is available, strains from its closest relative will have the highest probability of success in N fixation.

**Table 12.** Characters considered desirable for inoculant strains and inoculant carriers

**Strain characters for legume inoculants**

1. Ability to form nodules and fix N on the target legume
2. A wide host range, i.e. the ability to fix N with a wide range of host genotypes
3. Ability to fix N across a wide range of environmental conditions
4. Ability to compete in nodule formation with populations of rhizobia already present in the soil
5. Ability to form nodules and fix N in the presence of soil nitrate
6. Ability to grow well in artificial media, in inoculant carrier and in the soil
7. Low mortality on inoculated seed
8. Ability to migrate from the initial site of inoculation
9. Ability to tolerate environmental stress
10. Ability to colonise the rhizosphere of the host plant
11. Ability to colonise the soil in the absence of a legume host
12. Genetic stability
13. Compatibility with agrichemicals

**Properties of good inoculant carriers**

1. High water-holding capacity
2. Non-toxic to rhizobia
3. Easy to sterilise by autoclaving or gamma irradiation
4. Readily and inexpensively available
5. Sufficiently adhesive for effective application to seed
6. Good pH buffering capacity
7. Good cation- and anion-exchange capacities

Source: Thompson (1980), Keyser et al. (1992), Brockwell and Bottomley (1995), G. Bullard (pers. comm.)

There appears to be scope for selecting strains that are adapted to harsh conditions. For instance, Surange et al. (1997) identified acacia rhizobia that were tolerant of high salt concentrations and high alkalinity, and Kang et al. (1998) acid-tolerant strains.

### **Inoculant production**

There is a profuse literature on all aspects of large- and small-scale preparation of legume inoculant. The major papers on the principles and practice include Vincent (1970), Burton (1976, 1979, 1982), Brockwell (1977), Date and Roughley (1977), Thompson (1980, 1983), Williams (1984), Somasegaran (1985, 1991), Diem et al. (1989), Keyser et al. (1992), Smith (1992) and Somasegaran and Hoben (1994).

The first step in inoculant preparation is the production of a broth with, desirably, a population density of at least one billion ( $1 \times 10^9$ ) viable rhizobial cells per mL. The broth is grown in a fermenter which can be a very simple piece of equipment (Date and Roughley 1977). The broth is then incorporated in finely ground peat which preferably has been sterilised by gamma-irradiation (Roughley and Vincent 1967) or autoclaving. Finally, the peat culture is packaged and sealed into polyethylene bags of convenient size, 'matured' at 25°C for 14 days and stored, usually at about 4°C, until it is needed. The demand for acacia inoculant is likely to be met by small fermenters. Balatti (1982), Hoben and Somasegaran (1992) and Somasegaran et al. (1992) provide specifications for small fermenters, any of which would be suitable for the production of high-titre broths of strains of acacia rhizobia.

When commercial manufacture of legume inoculants began in the 1890s, liquid (broth) culture was the preferred form. Nowadays, the vast majority of inoculants are prepared in powdered organic carriers such as finely ground peat. Nevertheless, it is feasible to manufacture and market high-quality liquid inoculant (Lie et al. 1992; Brockwell et al. 1995a). Indeed, in their experiments described earlier, Galiana et al. (1994, 1996, 1998) used liquid inoculum for nursery-grown acacia seedlings that were then outplanted. Existing inoculants of highest quality tend to be those produced by small factories under the umbrella of a quality control authority, such as those described by Roughley and Pulsford (1982), Thompson (1983) and Somasegaran (1991). The NifTAL Center and MIRCEN in Hawaii have developed a 'micro-production unit' for small-scale production of rhizobial inoculants (NifTAL Center 1993). There are two key concepts: (i) use of a sterile peat carrier (cf. Date and Roughley 1977) and (ii) dilution of the rhizobial broth culture with sterile water (Somasegaran and Halliday 1982; Somasegaran 1985) immediately before it is injected into the sealed bag of peat carrier. The unit has a number of advantages over conventional (larger-scale) methods for producing legume inoculants. First, the unit is cheap and easy to assemble. Second, the number and size of fermentors used for propagating broth cultures of the rhizobia are reduced by using the dilution technique. Third, production schedules are very flexible. Fourth, because the unit uses sterile peat as a carrier, populations of rhizobia in the inoculant may be 10 times greater than in the many commercial inoculants manufactured from non-sterile carriers (cf. Roughley and Vincent 1967; Brockwell 2004).

Fifth, the use of sterile peat increases shelf-life over that of products made with non-sterile carriers. Sixth, the unit requires little space. Last, quality-control procedures are simple and reliable. NifTAL's micro-production unit appears ideal for preparing the relatively small quantities needed to meet the likely demand for acacia inoculants.

### **Multi-strain inoculants**

The current practice in Australia is to use a single effective strain of rhizobia in commercial legume inoculants (Roughley et al. 1984). Species-specific strains have been advocated for use in inoculants for certain *Acacia* species, e.g. *A. mearnsii* (Turk and Keyser 1992). The use of single-strain inoculants facilitates quality control (Thompson 1980), but has the major disadvantage of restricting the scope (host range) of any particular inoculant. It is common practice in most other countries to produce multi-strain inoculants. Somasegaran and Bohlool (1990) made an extensive comparison of multi- and single-strain inoculants. They found that, in almost all cases, the N-fixing effectiveness of a multi-strain inoculant exceeded or equalled the performance of the best strain in that inoculant. We therefore consider that use of multi-strain inoculants is essential to achieve successful nodulation and N fixation in acacias whenever it is proposed to use the one inoculant for more than one species.

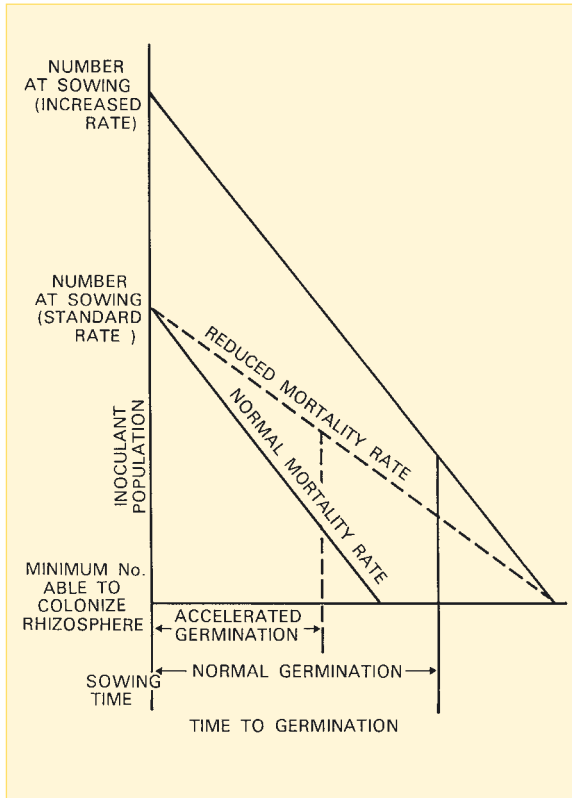
### **Inoculation strategy**

Although classified as free-living organisms with saprophytic properties (e.g. Chatel et al. 1968),

rhizobia are very much obligated to their hosts. Virtually all rhizobial multiplication in the wild takes place in the host rhizospheres, on the root surfaces and especially within the nodules. When an inoculant culture is applied to the seed surface or introduced into a seed bed or a nursery soil tube, the rhizobia start to die. The mortality continues until the development of a seedling rhizosphere that can be colonised by the surviving rhizobia. Initial procedures should therefore aim (i) to maximise survival of the inoculant during the non-rhizosphere period, and/or (ii) to accelerate germination so that the non-rhizosphere period is reduced. Figure 3 illustrates the principles involved.

For inoculation of acacias, the principles are easily implemented. Currently, most acacia plantings in the field are made using nursery stock raised in tubes of soil. Inoculant can be incorporated into the nursery soil immediately before planting the acacia seed. This general procedure was described in each of three reports of field benefits of inoculation: Lal and Khanna (1996) used seed inoculation, Galiana et al. (1994) applied liquid inoculum to the nursery soil, and Brockwell et al. (1999a) mixed peat culture into the soil. We believe that the last procedure, termed inoculation by soil enrichment, best meets the demands of the principles illustrated in Figure 3. Higher rates of inoculation can be achieved by incorporating an inoculant into the soil than by applying it to the seed surface, and experience has shown that peat-based inocula tend to survive better in soil and in the field than do liquid inocula (e.g. Brockwell 1962).





**Figure 3.** A schematic illustration of basic factors involved in improving the likelihood of legume nodulation following rhizobial inoculation: (i) reducing inoculant mortality, (ii) increasing rate of inoculant application, (iii) decreasing time to germination (after Brockwell 1962).

Accelerated germination of acacia seed can be effected by a pre-treatment of immersing the seeds in near-boiling water for a short time and then allowing them to imbibe the water as it cools.

Indeed, some operators actually boil their acacia seed for one minute. (Strictly speaking, with most acacia seed, the pre-treatment does more than merely accelerate germination; it triggers processes that stimulate germination.) Naturally, rhizobial enrichment of the soil should be done immediately before planting the seed. To avoid death of the inoculant from desiccation and/or heat stress, the soil should, after planting, be kept moist and as cool as practicable by the use of a misting device. It is pertinent that Gassama-Dia (1997), using delayed inoculation experiments, demonstrated that the period of maximum infectibility of *A. albida* inoculated with *Bradyrhizobium* was the 13 days immediately after seed germination.

A particular advantage of soil enrichment inoculation is that it lends itself to the preparation and use of inoculant strains selected for special purposes, e.g. tolerance of salinity, acidity, alkalinity and other forms of environmental stress.

There will be situations, as in large-scale land rehabilitation (cf. Thrall et al. 2001a,b), prevention of dryland salinisation and generation of carbon, conservation and biodiversity credits, when it is desirable to establish acacias by direct seeding. Alternative means of inoculation would be required in such circumstances. In studies of rhizobial survival following aerial seeding, Hely (1965) demonstrated that inoculation and seed coating produced vigorous swards of well-nodulated, N-fixing crimson clover (*Trifolium incarnatum*). It might be possible to develop a similar procedure for acacias.

### BREEDING AND SELECTION FOR ENHANCED ACACIA SYMBIOSIS

Because acacias are essentially non-domesticated plants, there must be some scope for breeding and selecting certain species for increased capacity to nodulate and fix N. We do not know, however, whether the sort of classical procedures outlined by Sprent (1994c) for an annual crop plant such as groundnut (*Arachis hypogaea*) would be applicable

to perennial tree and shrub species such as acacias. On the other hand, the simple, long-standing techniques for selecting for improved symbiosis in poorly domesticated forage plants that have been described by Hutton and Coote (1972) for greenleaf desmodium (*Desmodium intortum*) and by Zorin et al. (1976) for Caucasian clover (*Trifolium ambiguum*) might have application to acacias.



Alison Jeavons

*Acacia genistifolia*



## 6. Exploitation of the symbiosis

We have already pointed out that acacias are quite capable of existing in the absence of an effective symbiosis. There is no pretending that the quality of some acacia products, such as perfume oils and oyster poles, is influenced in any way by N fixation. However, sometimes it is difficult to separate cause and effect. In Australia, *A. harpophylla* is almost always associated with soils of high N status (Graham et al. 1981), but it is not possible to know with certainty whether the soil is high in N due to the presence of the acacia or if the acacia needs fertile soil to grow. Notwithstanding, there is no doubt that there are also many circumstances where the N fixed by acacias represents a valuable commodity.

### **NATURAL EXPLOITATION**

African peoples have long recognised that the extensively distributed, widely adapted indigenous leguminous tree, *A. albida*, confers special benefits on its immediate environment and, therefore, has a significant place in agricultural practice (Felker 1978; Giller and Wilson 1991; Saka et al. 1994). *Acacia tortilis* has similar status in the dry savannas of East Africa (Giller and Wilson 1991). These trees grow in the dry season and shed their leaves in the rainy season. A traditional farming practice in many African countries is the maintenance of parklands of

large *A. albida* trees, and to a lesser extent *A. tortilis*, in cultivated fields. Because the trees are deciduous in the rainy season, they do not compete for light with crop plants grown beneath them (cf. Giller and Wilson 1991). Also, *A. albida* may add substantial N to the soil as a result of the leaf fall that occurs at about the time that cash crops are sown. Indeed, in the Sahel, continuous dry-season cropping with sorghums and millets has been practised beneath *A. albida* without reductions in yields or additions of fertilisers (Porteres 1954). Yields of groundnut (*Arachis hypogea*) and cereals are often much higher under *A. albida* trees than in open fields (e.g. Charreau and Vidal 1965; Radwanski and Wickens 1967; Dancette and Poulain 1969; Charreau and Nicou 1971; Felker 1978; Poschen 1986). Moreover, the litter of *A. albida* and *A. tortilis* improves the soil environment by contributing to retention of soil moisture through an increase in soil organic matter, improving soil structure, enhancing populations of soil microfauna, and reducing extremes of evapotranspiration and soil temperature (e.g. Dancette and Poulain 1969; Bernhard-Reversat 1982; Young 1989). In addition to the N added to the soil as a result of leaf and pod fall, there may well be further supplementation of soil N through underground release of N in disintegrating, decaying roots and nodules. Much of all this is a consequence of the ability of *A. albida* and *A. tortilis*

to fix atmospheric N (see Table 4) although the trees might also have the capacity to access deep-soil N and N in groundwater. Additionally, both trees provide an abundance of fodder and fuelwood throughout the year and deep shade in the dry season (e.g. Bunderson et al. 1990). *Acacia albida* may be the Earth's most comprehensively utilised plant species and represents a classic example of natural exploitation of the legume symbiosis.

In both forest and savanna ecosystems, trees substantially influence the chemical and physical characteristics of the soil system through a variety of mechanisms, e.g. deposits of litter, the activity of soil macro- and microflora involved in litter decomposition, and redistribution and accumulation of soil nutrients through the scavenging and conduit properties of extensive root systems (Rhoades 1997). N-fixing trees make an additional, special contribution of N to the ecosystem and, consequently, other things being equal, represent a preferred form of vegetation in agroforestry. An apt example is the contribution made by *Acacia* species as natural understorey components of *Eucalyptus* forest ecosystems in Australia (Adams and Attiwill 1984; Hansen and Pate 1987a,b).

### GENERAL EXPLOITATION

There are many examples of successful exploitation of the genus *Acacia*, and its symbiosis with root-nodule bacteria. The endemic Hawaiian legume, *A. koa*, produces substantial biomass and fixes abundant N (Pearson and Vitousek 1997). Scowcroft and Jeffrey (1999) considered that *A. koa* has potential as a nurse crop to create understorey

conditions for the re-establishment of other components of Hawaiian highland forests cleared 150 years ago for pastoral use. The present rhizobial status of the soil, and therefore the need to inoculate the *A. koa*, is not known.

Acacias are used for rehabilitation of land damaged by industrial waste (e.g. Zhang et al. 1998), mining activities (e.g. Langkamp et al. 1979; Franco and de Faria 1997) and landfill (e.g. Chan et al. 1998), and for other forms of land reclamation (e.g. Kirmse and Norton 1984; Assefa and Kleiner 1998) including stabilisation of roadsides (e.g. Searle 1997). The genus has great scope for reclaiming saline land (Turnbull et al. 1998b). Coconut (*Cocos nucifera*) responded to interplanting with *A. auriculiformis* by increasing its total root biomass (Arachchi and Liyanage 1998). There is scope for using acacias to revegetate and rehabilitate degraded landscapes, such as the *Imperata* grasslands in Southeast Asia (Turnbull et al. 1998b), the Chilean secano interior (Arredondo et al. 1998), and the Atlantic lowlands of Costa Rica (Tilki and Fisher 1998). Temperate and tropical species of *Acacia* from Australia are suitable for many of these purposes.

### INTERCROPPING

Intercropping of a legume with a non-legume often increases significantly the amount of symbiotic N fixed by the legume and the total amount of N uptake by the joint components of the system — e.g. rice bean (*Vigna umbellata*)/maize (*Zea mays*) (Rerkasem et al. 1988); soybean (*Glycine max*)/maize (Martin et al. 1991); French bean (*Phaseolus vulgaris*)/maize (Pineda et al. 1994). It seems likely



that, at least partly, the uptake of soil N by the non-legume reduces the amount of soil N available to the legume, thereby 'forcing' it to fix more atmospheric N in order to fulfil its requirement for N. In some circumstances, however, intercropping might suppress legume yield when both plants compete for the same limited resources (e.g. Hakim et al. 1991).

Intercropping has been successfully applied to forestry. Turvey et al. (1983) reported that planted pine trees benefitted from an association with naturally regenerated *Acacia* species. The extent of the response was related to the density of the acacias. In experiments in plantations in Southeast Asia and Australia, Khanna (1997, 1998) grew mixed stands of fast-growing species of (tropical and temperate, respectively) *Acacia* and *Eucalyptus*. The *Acacia* species were actively fixing N. Their presence led to incremental growth in the *Eucalyptus* species (Table 13; Figure 4) which was

attributed to enhanced N status. A similar effect has been obtained with *A. mearnsii* and *Eucalyptus nitens* (shining gum) in south-eastern New South Wales (J. Brockwell, P.A. Mitchell and S.D. Searle, unpublished data).

Khanna (1998) postulated that the improvement was brought about by underground transfer of N from the *Acacia* to the *Eucalyptus*. He ruled out the possibility of above-ground transfer in his experiments because, at the time of increased *Eucalyptus* growth, decomposition of litter on the soil surface was trivial and had contributed little or nothing to the N dynamics of the system. The literature is equivocal on the question of underground transfer of legume N to associated non-legumes. There are arguments for — e.g. work and citations by Ofiri and Stern (1987); Viera-Vargas et al. (1995) — and against — papers cited by Peoples and Craswell (1992).

**Table 13.** Mean tree basal area (cm<sup>2</sup>/tree) in pure and mixed stands of *Eucalyptus globulus* and *Acacia mearnsii* grown at two densities for 45 months at Cann River, Victoria, Australia

Tree density (ratio)		Total tree density			
<i>Eucalyptus</i>	<i>Acacia</i>	1000/ha		1500/ha	
		<i>Eucalyptus</i>	<i>Acacia</i>	<i>Eucalyptus</i>	<i>Acacia</i>
100	0	9.1 b*	–	13.0 c	–
75	25	11.4 ab	41.4 a	15.9 ab	49.1 a
50	50	14.1 a	29.3 b	18.2 a	42.5 b
25	75	14.3 a	24.3 cd	17.3 a	36.5 c
0	100	–	19.8 d	–	25.2 d

Source: after P.K. Khanna, unpublished data.

\* In any one column, values with a common letter are not significantly different from one another ( $P > 0.05$ ).

Khanna's (1997, 1998) evidence for underground transfer of *Acacia* N to *Eucalyptus* is most convincing. In further studies involving fine-root architecture, researchers in Khanna's laboratory (Bauhus et al. 2000) found 'high' concentrations of N in the fine roots of *Eucalyptus globulus* (blue gum) when it was grown in a 50:50 mixture with *A. mearnsii*. This was another indication that the

improvement in the N nutrition of the eucalypts was a consequence of association with acacias.

It is interesting to speculate just how such transfers might occur. While trees are capable of using both nitrate and ammonium for growth (Devisser and Keltjens 1993; Turnbull et al. 1995), it seems unlikely that these products, released from the



**Figure 4.** Cartoon about the benefits of interplanting *Eucalyptus* and *Acacia* species (with acknowledgment to *The Canberra Times*)

organic N in root and nodule debris by classical nitrification processes, would have been available for underground transfer — for the same reasons that above-ground decomposition of litter was negligible. We favour a hypothesis centred on mycorrhizal activity. Extensive, diverse populations of mycorrhiza are extremely common in forest ecosystems. Although Khanna (1997, 1998) did not say so, there is the strong likelihood that his eucalypts were mycorrhizal. Mycorrhizal plants access N as free amino acids and, as well, are able to absorb N from proteins and chitins; see several references cited by Boddey et al. (2000b). These latter sources of N also are probably absorbed in the form of amino acid which has been made available by hydrolysis by mycorrhizal proteinases, chitinases and other enzymes (cf. Leake and Read 1989, 1990).

We submit that, in forest ecosystems, underground transfer of N from N-fixing trees to non-leguminous trees is mediated by mycorrhizal pathways. This process seems more likely to be of particular significance early in the development of the ecosystem before other processes of N dynamics become fully active.

### **LAND RECLAMATION: PRINCIPLES AND PRACTICE — THE AUSTRALIAN EXPERIENCE**

This section is based largely on recent, mainly unpublished experiences of Alison Jeavons and Meigan Waayers and colleagues in the North Central Catchment Management Authority and the Department of Primary Industries, Victoria. The observations have emerged from a series of

case studies and, taken altogether, represent the conventional wisdom on the use of *Acacia* species in land reclamation in southern Australia.

### **General principles**

Most undisturbed or lightly disturbed Australian landscapes, from the tropics and warm and cool temperate zones to the arid regions, encompassing rainforest, woodlands and pastoral country, have acacias as a component of their understoreys or, somewhat less frequently, as dominant species. It follows, therefore, that acacias are widely considered as suitable species for revegetation of lands that have become degraded due to inappropriate land use, salinity, erosion, waterlogging and other factors leading to loss of biodiversity. It follows also that, because of the wide diversity and distribution of the genus, there is almost invariably one or more *Acacia* species appropriate for any particular revegetation situation, with the possible exception of highly saline soils.

*Acacia* planting has been recognised as an effective tool for erosion control since the early 1960s because, in general, acacias can be readily established on degraded land. At that time, the choice of species was most often determined merely by seed availability, readiness of germination and tolerance of adverse environmental conditions, with little concern for other principles of acclimation. An unfortunate consequence was that some of the most easily established, vigorous species, e.g. *A. baileyana* and *A. longifolia*, became serious weeds outside their natural range. Nowadays, revegetation practitioners try to establish as many species as

practicable, with special emphasis on the use of local species and provenances of species, especially in undertakings directed at restoration of biodiversity. What precisely should be regarded as 'local' is hotly debated. There is a vocal body of opinion that specifies that seed should be sourced no more than 5 km from the intended point of revegetation. A much less restrictive definition is that any species is suitable for revegetation provided that it is not grown outside its natural range and that its seed is obtained from sites with the same soil type as the revegetation site and with similar rainfall. Research on these and related matters is currently in progress (A.G. Young, pers. comm.).

### General practice

There are two distinct methods of establishing acacias in revegetation undertakings: outplanting tube stock and direct seeding. Each has its advantages and disadvantages, summarised in Table 14. The major difference between the methods

relates to practicality. Outplanting tube stock grown in a nursery into the field is convenient and ideal for a small number of seedlings. Indeed, outplanting is the preferred option for getting greatest value from limited seed supplies. However, once the area to be sown exceeds 2–3 hectares, economies of scale favour direct seeding.<sup>4</sup> To give a general example, the North Central Native Vegetation Plan of the North Central Catchment Management Authority estimates that approximately 12,000 hectares of revegetation needs to be undertaken every year for the next 20 years in order to reverse the regional decline in biodiversity. Outplanting is clearly not feasible for so vast an undertaking. A more specific example relates to a revegetation project for salinity control carried out on the Big Hill range south of Bendigo, Victoria. This project saw the establishment of about one million trees and shrubs across nearly 1000 hectares of high recharge hills.

<sup>4</sup> Custom-built equipment for direct seeding has been described by Dalton (1990) and Scheltema (1992).



Peter Thrall

The retention of vegetation, including species of *Acacia*, *Eucalyptus* and *Allocasuarina*, preserves the integrity of streamlines.

## NITROGEN FIXATION IN ACACIAS

The bulk of the work was carried out through direct seeding, for two months in each of two years, and involved three or four people. The cost of labour and materials was less than \$100,000. Had the project

been attempted by outplanting nursery-grown tube stock, it would have required the services of 65 people for five days each week for four months, and the total cost would have exceeded \$2,000,000.



Courtesy of the Australian National Botanic Gardens

The banks of this ephemeral watercourse have been seriously eroded following removal of vegetation. Further erosion can be readily contained by revegetating with surface-sown native species including acacias.



Alicia Jeavons

Three-year-old roadside revegetation with acacias established by direct seeding



Peter Thrall

This creek side, near St Arnaud, Victoria, was at risk because understorey shrubs had disappeared. It has recently been revegetated by surface-sowing with native species, including acacias inoculated with effective strains of root-nodule bacteria.

**Table 14.** Comparison of the advantages and disadvantages of (i) direct seeding and (ii) outplanting of nursery-grown tube stock as means for establishment of acacias and other Australian native trees and shrubs to reclaim degraded land

	<b>Direct seeding</b>	<b>Outplanting of nursery-grown tube stock</b>
<b>Advantages</b>	Substantially lower costs than for other methods of planting	Time tested for reliable results
	High densities of established plants are attainable	Technology is well known and accepted
	Random plant distribution is attainable where a natural 'look' is required	Appropriate where there is a need for fixed plant density, uniform spacing or a particular species in a particular place
	Mature plants are more wind stable — because root systems have never been disturbed	Always slightly ahead in early growth compared with direct seeding; therefore useful when a quick effect is needed
	When using a wide range of species, savings on propagation and planting far outweigh costs of additional seed	Supplements direct seeding where gaps occur in the plant stand
	<b>Direct seeding</b>	<b>Outplanting of nursery-grown tube stock</b>
<b>Disadvantages</b>	Low rainfall, seasonal variation, erodable or heavy clay soil, insect predation and weed competition are all more critical for direct seeding than for outplanting	Considerably higher costs and much more labour intensive, especially on a broad scale, than for direct seeding
	Sometimes seedling establishment is patchy and gaps need filling	Only feasible for small-scale projects (probably never exceeding 5 ha)
	Greater quantities of seed required than for nursery-grown seedlings	
	Limited to species that germinate readily from (treated) seed	

Source: derived from Dalton (1990).

## NITROGEN FIXATION IN ACACIAS



Courtesy of the Australian National Botanic Gardens

The integrity of this erosion-prone hillside on the Eyre Peninsula, South Australia, has been preserved by a stand of *Acacia pycnantha* (golden wattle).



Peter Thrall

The use of a knockdown herbicide allows surface-seeded acacias to establish without competition from weeds.



Bob Gault

Germination of acacia seed sown into the field by direct seeding is enhanced by application of smoked water into the seed furrow. This picture, showing seed and water delivered on to a concrete surface, is a simulation of the procedure.



John Brockwell

Using direct seeding to establish salt-tolerant acacias on salinity-endangered land at Bald Rock, Victoria. The seeder shown here is a modification of the one described by Dalton (1990).

## General considerations

### *Availability of acacia seed*

A significant constraint to the success of direct seeding is seed availability. As a rule, acacia seed is easily collected from the wild. However, with many species, the quantities of seed required for direct seeding of degraded land (up to 0.5 kg per hectare) often greatly outstrip the amounts available from good quality remnant stands without drastic depletion of resources necessary for sustaining populations of wildlife, especially birds. This is a particular problem in those circumstances where local provenances are considered essential for effective revegetation. As an example, the North Central Catchment Management Authority has a requirement for its revegetation program of 6000 kg acacia seed per year. Although the Authority has an exceptionally good group of seed collectors, they manage to collect only about 500 kg seed per year from within the catchment. The mining industry also has a large demand for seed for restoration of mine sites where seeding rates may be up to 35 kg per hectare. All this is reflected in the cost of commercially available acacia seed — \$120–\$510 per kg (Australian Seed Company 2003). Certain Australian organisations with responsibility for land reclamation are now tackling these problems through extension and training programs, one result being the establishment in recent years of a number of acacia seed orchards.

### *Seed germination*

Acacia seed usually has high viability but is notoriously hard-seeded. Under natural conditions,

except following exposure to fire, it tends to germinate slowly, intermittently and over a long period. Often only 10–40% of viable seed establishes beyond the seedling stage (Dalton 1990). Effective establishment, for both direct seeding and tube stock, requires seed treatment to enhance germinability. Immersion in boiling water (the seed imbibes while the water is cooling) and mechanical scarification are efficient means for dealing with the problem of hard seed. The use of smoked water, either as a supplementary seed treatment or delivered directly into the seedbed alongside the seed, further enhances germination. A butenolide has been identified as the compound in smoked water that stimulates seed germination (Flematti et al. 2004).

### *Miscellaneous constraints to acacia seedling establishment*

Naturally, seedling establishment of direct-seeded acacias is best under good seasonal conditions. Nonetheless, good results have sometimes been achieved during drought.

Even with good germination following seed treatment, early growth of direct-seeded acacias is relatively slow and its continued success is dependent on effective control of first-year competition from annual weeds. It is standard practice to seed directly into a furrow placed centrally along a strip of land from which weeds have been eliminated with a knock-down herbicide. In addition to their competitive effect, some annual weeds harbour insect pests, e.g. red-legged earth mite *Halotydeus destructor* (Tuck.) (Acarina:





Eurodidae) on capeweed (*Arctotheca calendula*), which feed on acacia seedlings.

Herbivores, in particular rabbits, are partial to acacia seedlings. Wherever large rabbit populations exist in the vicinity of revegetation sites they must be controlled .

### **The role of the acacia symbiosis in land reclamation**

Many reclamation projects involving acacias are aimed at degraded land that once carried acacias as an understorey component or sometimes as the dominant species. The soils of such land, almost certainly once contained populations of acacia rhizobia which nodulated and fixed N with their hosts. However, with the disappearance of the acacias, it appears that their rhizobia have also been lost from the soil (Thrall et al. 2001b). Restoration of healthy acacia communities therefore involves the reintroduction of effective rhizobia as well as the acacias themselves. Recent research work (Thrall et al. 2005) has shown that rhizobial inoculation (by seed coating) of direct-seeded acacias more than doubles their establishment rate and often significantly increases seedling growth rates.

An immediate consequence of these findings on seedling establishment is that it becomes possible to reduce direct seeding rates by at least half, reducing costs and the demand for seed. Moreover, increased

early growth may well assist acacia seedlings to outrun first-year competition from weeds. This is an especially important consideration in revegetating riparian zones which, in Australia, are among the most endangered ecosystems. A further benefit of rapid early growth is that it is likely to make acacia seedlings more tolerant of grazing by macropods (wallabies, kangaroos) and/or feral animals (rabbits, hares).

Application of inoculant to acacia seed by seed coating is a time-consuming process, especially where several seed lots require inoculation, and may constitute a bottleneck at seeding time. A possible alternative is a free-flowing granular inoculant (cf. Scudder 1975; Brockwell et al. 1980). This product, applied directly into the seed bed through a hopper attached to the seeding equipment, has been used successfully in the United States for inoculation of soybean and peanut crops (Scudder 1975). A similar device might be useful for direct seeding of acacias.

It seems reasonable to conclude that proper exploitation of the symbiosis might reduce the cost of acacia establishment in revegetation undertakings. Although exact costs are not yet known, it is estimated that a 50% reduction in seed usage, combined with application of granular inoculant directly into the seed bed, would reduce costs by at least 20–25%.



## 7. General conclusions and prognosis

The body of literature relating to the symbiosis between the genus *Acacia* and its diverse rhizobia that we have reviewed is dispersed in terms of the species investigated, the aspects of the symbiotic relationship studied and the places of publication of results. There appear to be three main reasons for this: first, the genus is so large — some 1350 species; second, it is more difficult to experiment with shrubs and trees than it is with herbs; third, it has been unfashionable until recently to work with N-fixing trees. With respect to the third reason, the publication of newsletters such as *NFT News: Improvement and Culture of Nitrogen Fixing Trees* (published by IUFRO and CSIRO Forestry and Forest Products, Canberra) and *Farm Forestry News* (once published by the Forestry/Fuelwood Research and Development (F/FRED) Project, c/o Winrock International: Arlington, VA — publication now ceased) has created forums for exchange of experience, views and ideas and has done much to overcome any resistance that might once have existed against working with N-fixing trees. As the reference list shows, the period since 1990 has seen a proliferation of research and extension papers dealing with aspects of the symbiosis of acacias.

It became clear to us during the review of the literature that the processes of root nodulation and

N fixation in the genus *Acacia*, and the procedures for studying them, are generally little different from those of other, more extensively studied legume genera. Accordingly, where there appeared to be no information about particular aspects of acacia symbiosis, we drew upon experience with other N-fixing plants.

### THE PLANT

*Acacia* is one of the largest genera of flowering plants, widely distributed mainly in Australia, Africa and Asia and with a multitude of products extensively utilised by humans and wildlife. The genus is adapted to growth on many types of soil, including those of low fertility, and is tolerant of arid and semi-arid environments. It is a prominent component of many forest ecosystems and, as a N-fixing legume, apparently contributes substantially to natural N cycling. Although millions of hectares of land have been planted with acacias, especially for plantation and farm forestry and rehabilitation of degraded landscapes, many authorities consider that the genus is underutilised. The literature that we surveyed gave us the very strong impression that the symbiosis of acacias with root-nodule bacteria, and their capacity to fix atmospheric N as a consequence of that association, is also underutilised.

## THE BACTERIA

The rhizobia that form root nodules on acacias appear to be at least as widely distributed as the plants themselves. Indeed, rhizobia capable of nodulating and fixing N with species of *Acacia* can often be found in soils in which no acacias are growing. Such organisms associate with legumes that are symbiotically related to *Acacia* species. There is a small taxonomic group of *Acacia* that is known to be non-nodulating. Otherwise, reports of failures of nodulation in natural ecosystems should be regarded cautiously. Lack of nodulation is more likely to be due to harsh environmental conditions than to absence of rhizobia from the soil.

There are diverse groups of acacia rhizobia, including fast-growing, slow-growing and very-slow-growing types. Organisms that form nodules on various acacias are certainly represented in at least four of the currently recognised genera of rhizobia. This number may be further expanded as the systematics of the Rhizobaceae becomes clearer.

It is inevitable with such diverse symbionts that host/bacterial relationships should exhibit complex interactions leading to specificity in both bacterium and plant in terms of nodule formation and N fixation. While there are very many strains of rhizobia that are able to nodulate many species of *Acacia*, the symbioses are often ineffective or poorly effective in fixing N. In other words, many but not all *Acacia* species have a requirement for specific rhizobial strains in order to express their capacity to fix atmospheric N. This characteristic has implications for the selection of strains of rhizobia for preparation of acacia inoculants. An

important example of an acacia that appears highly specific is *A. mangium*. This species is native to northern Australia and Papua-New Guinea where it apparently nodulates and fixes N with resident strains of rhizobia. Where it has been introduced into the tropics of the northern hemisphere, its N fixation often appears to be impaired by the absence of specific rhizobia.

## THE SYMBIOSIS

There are numerous reports of measurements of N fixation by *Acacia* species. Several mensuration procedures were used and the data that emerged are extremely variable — from virtually zero up to 200 kg per hectare per annum. There is no suggestion that the variability was an artifact of method, but some of the differences may have been a consequence of poorly effective or ineffective symbioses. Some of the reports allowed a comparison of the amounts of N fixed by acacias and by other N-fixing trees. Generally, *Acacia* fared poorly compared with genera such as *Calliandria*, *Gliricidia* and *Leucaena*. An examination of the circumstances revealed that the two data sets were not strictly comparable. Most measurements of acacia N fixation had been made in natural ecosystems whereas the N fixed by other tree legumes had been measured in anthropogenic ecosystems. There is a wealth of literature reporting substantial differences in the N dynamics of the two systems. In natural ecosystems, N cycling takes place at a relatively rapid rate aided by diverse macro- and microflora that decompose leaf litter above ground and root fragments and non-living components of the soil community underground.

The organic N released by these processes is transformed to nitrate by nitrifying organisms. It is well known that nitrate is an inhibitor of legume nodulation and N fixation. Where natural forest has been cleared for agriculture or silviculture, N cycling is much reduced, with lower rates of nitrification and lower levels of soil nitrate. In the circumstances of these anthropogenic ecosystems, it is only to be expected that legume N fixation would be inhibited less than in natural ecosystems. We have concluded from these considerations, and despite the superficial evidence to the contrary, that there is good reason to believe that the intrinsic N-fixing capacity of acacias is just as great as for other tree legumes.

The different elements of naturally occurring populations of acacia rhizobia were extremely variable in their N-fixing effectiveness, even in association with *Acacia* species that grew in the immediate vicinity. Some highly effective strains have been isolated that showed their superiority in glasshouse tests. There were very few reports of strain effectiveness trials in the field. The most compelling of these were the works of Galiana and colleagues (Galiana et al. 1990, 1994, 1996, 1998) in West Africa. They demonstrated impressive growth responses to inoculation of *A. mangium* with effective strains of rhizobia, as well as longevity of the inoculant in the field. The inoculation was performed by sowing seed into nursery soil augmented with rhizobial culture. The seedlings that emerged were well nodulated and continued to fix N when they were outplanted into the field. The procedure was termed inoculation by soil enrichment and has been exploited by others.

## EXPLOITATION

Most of the world's acacias grow in forests or woodlands. For reasons of microbial ecological competition with populations of acacia rhizobia already resident in those soils, the chances of successfully and permanently introducing more-effective strains are nil. So there is probably little that can be done to increase the N fixation by acacias in natural ecosystems, except to improve their vigour. It is a rule of thumb (Peoples et al. 1995a, b), perhaps somewhat oversimplified, that the greater a legume's biomass the more N it fixes. Applying this principle, correction of nutrient deficiencies and control of insect pests, for instance, are legitimate means of increasing the vigour of acacias and, therefore, the amount of their atmospheric N fixation. Economic considerations would determine the practicalities of such an approach. There is one circumstance where such a course of action merits consideration. Following timber harvesting or wildfire in ecosystems where they occur, acacias are frequently the primary recolonising species and may be dominant for many months or even several years. Any strategy that might be used at this time to increase their N fixation would be of subsequent benefit to non-N-fixing components of the ecosystem. An appropriate strategy would also assist in the replenishment of the total pool of soil N which occurs when the quantity of N removed as plant (or animal) product is less than the amount of fixed atmospheric N that remains behind in legume residues.

Where acacias are used in plantation and farm forestry, there is a great untapped potential for exploiting the symbiosis. Nearly all plantation and



farm trees (and shrubs) are outplanted as nursery stock. When soil enrichment inoculation is applied to nursery soil immediately before seeding, the large population of effective inoculant rhizobia in the root medium leads to prompt nodulation of the young acacia seedlings and early onset of N fixation. Seedlings that have been inoculated reach planting size more quickly than uninoculated seedlings and, therefore, require less time in the nursery. When the vigorous seedlings that result are outplanted into the field, their survival and early growth are better than those of uninoculated seedlings. These advantages may persist for some time and may lead to early closure of canopy and lower maintenance costs. In addition, the effective inoculant strains persist in the soil for several years where they would remain a continuing potent source of inoculant for the infection and nodulation of new roots.

The principles applying to successful inoculation (Figure 3) suggested that peat culture, because it appears to have a rather slower rate of mortality in storage than liquid culture, may be the preferred form of acacia inoculant for use in commercial nurseries. Indeed, peat inoculant is already used in Australian plant nurseries that produce large numbers of acacia seedlings as tube stock (Brockwell et al. 1999a). It was clear, for reasons of host/rhizobia specificity, that a single strain of rhizobia could never be expected to nodulate and fix N with the complete range of *Acacia* species in demand for plantation and farm forestry. Thus, it will be necessary to produce acacia inoculant that comprises several strains that, between them, are effective in N fixation for the range of *Acacia* species raised in nurseries.

This is not a problem in most parts of the world, where manufacture of mixed-strain legume inoculants is very often standard practice. In Australia, however, where there is a policy of single-strain inoculants, it would be necessary to have packets of inoculant containing several individual culture packs and for the user to mix the contents immediately before nursery soil inoculation by soil enrichment. Because demand for acacia inoculant is likely to be quite limited, it might be a problem to find manufacturers willing to produce small quantities of the specialised inoculant. The extent of demand can be illustrated by a simple calculation. At a conservatively sensible rate of soil enrichment inoculation, one kilogram of inoculant is enough for 10,000 acacia seedlings. This in turn is sufficient for 10 hectares of plantation. Thus, the total requirement for a large planting of 1000 hectares is just 100 kg of inoculant. The very low cost of soil enrichment inoculation of nursery-grown acacia tube stock (a fraction of a cent each, which seems a small price to pay for increased vigour, survival and growth of seedlings outplanted into the field), may be a two-edged sword. One means of dealing with the problem would be for nurseries to maintain their own stocks of acacia inoculant. Well-nodulated, N-fixing, diverse species of *Acacia* could be grown together in an 'inoculant' plot, the soil from which could later be used to enrich soil intended for the production of tube stock. Unfortunately for this proposal, some authorities legislate against the use of non-sterile soil in plant nurseries. A rather unsatisfactory solution to the problem suggested by Masutha et al. (1997) was to use only those (few) species of *Acacia* that are sufficiently promiscuous in their requirements for effective rhizobia that

## NITROGEN FIXATION IN ACACIAS

they are likely to nodulate vigorously and fix N abundantly with whatever populations of rhizobia occur naturally in soil.

As a result of our literature review, we are convinced that there is considerable potential worldwide and especially in Africa, Asia and Australia to enhance the vigour, N-fixing capacity and productivity of the many *Acacia* species by more fully and efficiently exploiting their symbioses with root-nodule bacteria. Simultaneously, improved symbioses

would augment resources of soil N. For many species this will involve inoculation with effective rhizobia. There may also be scope, as suggested by Smith and Daft (1978), for dual inoculation with rhizobia and mycorrhizal fungi to remove other nutritional constraints. Even in circumstances where inoculation is not practicable and/or feasible, the cultivation of acacias has potential to enhance soil fertility and soil structure in plantations, on farms and in land-rehabilitation projects.



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*Acacia mearnsii*

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- <sup>5</sup> Entries marked with an asterisk are citations from the program and abstracts of the Fourth International Legume Conference held in 2001. There will be no composite publication of the proceedings of that conference. However, it is intended that individual contributions will be published elsewhere. Some have already appeared in Klitgaard and Bruneau (2003), and in *Biochemical Systematics and Ecology*, 31(8), 2003.
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## NITROGEN FIXATION IN ACACIAS

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# Appendix

In the text and tables, (i) acacias have been referred to by their botanical names (genus and species) whereas (ii) other plants have been referred to by their botanical names and (where known) their common names. Mostly for acacias and sometimes for other plants, the generic name has been abbreviated to an initial. Table A1 gives the botanical names of all the plants that we have

mentioned, with the authorities for those names. The addition of the authority allows the plants to be identified unambiguously. It is customary to italicise botanical names but not authorities. Common names are also given. There is no 'correct' common name for a plant. Where more than one common name is given, the one listed first is merely our own preference.

**Table A1.** Botanical and common names<sup>a</sup> referred to in this review. The first common name listed is the preferred common name.

Botanical name and authority	Common name(s)
<b>Acacias</b>	
<i>Acacia abyssinica</i> Benth	Not known <sup>a</sup>
<i>Acacia acuminata</i> Benth.	Raspberry jam, jam wattle
<i>Acacia alata</i> R. Br.	Winged wattle
<i>Acacia albida</i> Del. <sup>b</sup>	Applering acacia, ana tree, winter thorn
<i>Acacia ampliceps</i> Maslin	Salt wattle
<i>Acacia aneura</i> F. Muell ex Benth.	Mulga
<i>Acacia arabica</i> (Lam.) Willd. <sup>c</sup>	Prickly acacia, babul
<i>Acacia ataxacantha</i> DC.	Not known
<i>Acacia aulacocarpa</i> A. Cunn. ex Benth.	Brush ironbark, hickory wattle
<i>Acacia auriculiformis</i> A. Cunn. ex Benth.	Earpod wattle
<i>Acacia bahiensis</i> Benth.	Not known
<i>Acacia baileyana</i> F. Muell.	Cootamundra wattle
<i>Acacia berlandieri</i> Benth.	Berlandia acacia, guajillo
<i>Acacia brevispica</i> Harms	Not known
<i>Acacia cambagei</i> R.T. Baker	Gidgee, stinking wattle
<i>Acacia catechu</i> (L.f.) Willd.	Catechu, black cutch
<i>Acacia caven</i> (Molina) Molina	Espino caven



**Table A1.** (cont'd) Botanical and common names<sup>a</sup> referred to in this review. The first common name listed is the preferred common name.

Botanical name and authority	Common name(s)
<b>Acacias (cont'd)</b>	
<i>Acacia cincinnata</i> F. Muell.	Scorpion wattle
<i>Acacia confusa</i> Merr.	Not known
<i>Acacia crassicarpa</i> A. Cunn. ex Benth.	Lancewood
<i>Acacia cyanophylla</i> Lindl. <sup>d</sup>	Golden wreath wattle
<i>Acacia cyclops</i> A. Cunn. ex G. Don	Western coastal wattle
<i>Acacia dealbata</i> Link	Silver wattle
<i>Acacia decurrens</i> Willd.	Green wattle, early black wattle
<i>Acacia difficilis</i> Maiden	Not known
<i>Acacia elata</i> A. Cunn. ex Benth.	Mountain cedar wattle, cedar wattle
<i>Acacia erioloba</i> E. Meyer	Not known
<i>Acacia excelsa</i> Benth.	Ironwood, rosewood, ironwood wattle
<i>Acacia farnesiana</i> (L.) Willd.	Mimosa bush, sweet wattle
<i>Acacia fleckii</i> Schinz.	Not known
<i>Acacia genistifolia</i> Link	Spreading wattle, early wattle
<i>Acacia gerrardii</i> Benth.	Not known
<i>Acacia glomerosa</i> Benth.	Not known
<i>Acacia greggii</i> A. Gray	Catclaw acacia, Texas mimosa
<i>Acacia harpophylla</i> F. Muell. ex Benth.	Brigalow
<i>Acacia hebeclada</i> DC.	Not known
<i>Acacia hereroensis</i> Engl.	Not known
<i>Acacia hilliana</i> Maiden	Not known
<i>Acacia holocericea</i> A. Cunn. ex G. Don	Candelabra wattle
<i>Acacia homalophylla</i> A. Cunn. ex Benth.	Yarran
<i>Acacia imbricata</i> F. Muell.	Imbricate wattle
<i>Acacia implexa</i> Benth.	Lightwood, hickory wattle
<i>Acacia irrorata</i> Sieber ex Spreng.	Green wattle
<i>Acacia julifera</i> Benth.	Not known
<i>Acacia karroo</i> Hayne	Karoo thorn
<i>Acacia kempeana</i> F. Muell.	Witchetty bush
<i>Acacia kirkii</i> Oliver	Not known
<i>Acacia koa</i> A. Gray	Koa acacia, koa
<i>Acacia leptocarpa</i> A. Cunn. ex Benth.	Not known
<i>Acacia leucophloea</i> (Roxb.) Willd.	Nimbar
<i>Acacia leucophylla</i> Lindl.	Not known

**Table A1.** (cont'd) Botanical and common names<sup>a</sup> referred to in this review. The first common name listed is the preferred common name.

Botanical name and authority	Common name(s)
<b>Acacias (cont'd)</b>	
<i>Acacia littorea</i> Maslin	Western Australian coastal dune wattle
<i>Acacia longifolia</i> (Andrews) Willd.	Sydney golden wattle, sallow wattle
<i>Acacia macrostachya</i> DC.	Not known
<i>Acacia maidenii</i> F. Muell.	Maiden's wattle
<i>Acacia mangium</i> Willd.	Brown salwood, hickory wattle
<i>Acacia martii</i> Benth.	Not known
<i>Acacia mearnsii</i> De Wild.	Black wattle, green wattle
<i>Acacia melanoxylon</i> R. Br.	Blackwood, Tasmanian blackwood
<i>Acacia mellifera</i> (M. Vahl.) Benth.	Not known
<i>Acacia mucronata</i> Willd. ex H.L. Wendl.	Narrow-leaved wattle, variable sally
<i>Acacia neriiifolia</i> A. Cunn. ex Benth.	Silver wattle, oleander wattle
<i>Acacia nigrescens</i> Oliver	Knob thorn
<i>Acacia nilotica</i> (L.) Del.	Prickly acacia, babul
<i>Acacia notabilis</i> F. Muell.	Hickory wattle
<i>Acacia oxycedrus</i> Sieber ex DC.	Spike wattle
<i>Acacia papyrocarpa</i> Benth.	Western myall
<i>Acacia paradoxa</i> DC.	Kangaroo thorn
<i>Acacia parramattensis</i> Tindale	Parramatta wattle, green wattle
<i>Acacia pellita</i> O. Schwarz.	Not known
<i>Acacia pendula</i> A. Cunn. ex G. Don	Weeping myall, boree, myall
<i>Acacia pennata</i> (L.) Willd.	Not known
<i>Acacia penninervis</i> Sieber ex DC	Mountain hickory
<i>Acacia pentogona</i> (Schum.) Hook. f.	Not known
<i>Acacia peuce</i> F. Muell.	Waddywood
<i>Acacia plectocarpa</i> A. Cunn. ex Benth.	Not known
<i>Acacia podalyriifolia</i> A. Cunn. ex G. Don	Queensland silver wattle, Mt Morgan wattle
<i>Acacia polyacantha</i> Willd.	Not known
<i>Acacia polyphylla</i> DC.	Not known
<i>Acacia pulchella</i> R. Br.	Prickly Moses
<i>Acacia pycnantha</i> Benth.	Golden wattle, broad-leaved wattle
<i>Acacia raddiana</i> Savi <sup>c</sup>	Umbrella thorn
<i>Acacia redolens</i> Maslin	Ongerup wattle
<i>Acacia reficiens</i> Wawra	Not known
<i>Acacia retinodes</i> Schldl.	Wiralda, swamp wattle

**Table A1.** (cont'd) Botanical and common names<sup>a</sup> referred to in this review. The first common name listed is the preferred common name.

Botanical name and authority	Common name(s)
<b>Acacias (cont'd)</b>	
<i>Acacia salicina</i> Lindley	Cooba, native willow, doolan
<i>Acacia saligna</i> (Labill.) Wendl. <sup>d</sup>	Golden wreath wattle
<i>Acacia schweinfurthii</i> Brenan & Exell	Not known
<i>Acacia senegal</i> (L.) Willd.	Gum arabic, kher, senegal gum
<i>Acacia seyal</i> Del.	Shittimwood, talh, thirty-thorn
<i>Acacia signata</i> F. Meull.	Not known
<i>Acacia silvestris</i> Tind.	Bodalla silver wattle, red wattle
<i>Acacia smallii</i> Isely <sup>f</sup>	Huisache
<i>Acacia stenophylla</i> A. Cunn. ex Benth.	Eumong, river cooba
<i>Acacia suaveolens</i> (Smith) Willd.	Sweet wattle, sweet-scented wattle
<i>Acacia terminalis</i> (Salisb.) J.F. Macbr.	Sunshine wattle, New Year wattle
<i>Acacia tetragonophylla</i> F. Muell.	Dead finish, kurara
<i>Acacia tortilis</i> (Forsskal) Hayne <sup>e</sup>	Umbrella thorn
<i>Acacia trachycarpa</i> Pritz.	Sweet-scented minnie-ritchie
<i>Acacia trachyphloia</i> Tind.	Golden feather wattle
<i>Acacia tumida</i> F. Muell. ex Benth.	Pindan wattle
<i>Acacia verniciflua</i> Cunn.	Varnish(ed) wattle, manna wattle
<i>Acacia victoriae</i> Benth.	Prickly wattle, elegant wattle, gundabluie
<i>Faidherbia albida</i> (Del.) A. Chev. <sup>b</sup>	Applering acacia, ana tree, winter thorn
<b>Other leguminous trees</b>	
<i>Aotus ericoides</i> (Vent.) G. Don	Not known
<i>Cassia siamea</i> Lam. <sup>g</sup>	Djoowar, kassod-tree, Siamese senna
<i>Cassia spectabilis</i> DC. <sup>g</sup>	Not known
<i>Chamaecytisus proliferus</i> (L.f.) Link	Tree lucerne
<i>Leucaena esculenta</i> (Mocino & Sesse ex DC.) Benth.	Not known
<i>Leucaena leucocephala</i> (Lam.) De Wit	Leucaena, ipil-ipil, leadtree, jumbie bean
<i>Mimosa affinis</i> Harms ex Glaz.	Not known
<i>Parkia biglobosa</i> (Jacq.) R. Br. ex G. Don	African locust bean, nitta
<i>Prosopis chilensis</i> (Molina) Stuntz	Algarrobo, Chilean algarrobo
<i>Senna siamea</i> (Lam.) H. Irwin & Barneby <sup>g</sup>	Djoowar, kassod-tree, Siamese senna
<i>Senna spectabilis</i> (DC.) H. Irwin & Barneby <sup>g</sup>	Not known
<i>Tamarindus indica</i> L.	Tamarind

**Table A1.** (cont'd) Botanical and common names<sup>a</sup> referred to in this review. The first common name listed is the preferred common name.

Botanical name and authority	Common name(s)
<b>Other legumes</b>	
<i>Abrus precatorius</i> L.	Not known
<i>Aeschynomene indica</i> L.	Kat sola
<i>Amorpha fruticosa</i> L.	False indigo, indigo bush, bastard indigo
<i>Amphicarpaea trisperma</i> Baker	Not known
<i>Arachis hypogea</i> L.	Peanut, groundnut, goober, mani
<i>Aspalathus carnosa</i> P.J. Bergius	Not known
<i>Astragalus adsurgens</i> Pallas	Not known
<i>Astragalus sinicus</i> L.	Chinese milk vetch
<i>Cajanus cajan</i> (L.) Millsp.	Pigeonpea
<i>Coronilla varia</i> L. <sup>h</sup>	Crown vetch
<i>Cicer arietinum</i> L.	Chickpea, garbanzo
<i>Daviesia ulicifolia</i> C.R.P. Andrews	Gorse bitter pea
<i>Desmodium intortum</i> (Miller) Fawc. & Rendle	Greenleaf desmodium
<i>Desmodium sinuatum</i> Blume ex Baker	Not known
<i>Galega officinalis</i> L.	Goats-rue, galega
<i>Galega orientalis</i> Lam.	Not known
<i>Glycine max</i> (L.) Merr.	Soybean
<i>Glycine soja</i> Siebold & Zucc.	Wild soybean
<i>Glycine wightii</i> (Wight & Arn.) Verdc. <sup>i</sup>	Perennial glycine, creeping glycine
<i>Gueldenstaedtia multiflora</i> Bunge	Not known
<i>Hardenbergia violacea</i> (Schneev.) Stearn	False sarsaparilla
<i>Kennedia prostrata</i> R. Br.	Running postman
<i>Lotononis bainesii</i> Baker	Lotononis
<i>Medicago polymorpha</i> L.	Common burr medic, California bur clover
<i>Medicago ruthenica</i> (L.) Trautv.	Not known
<i>Medicago sativa</i> L.	Lucerne, alfalfa
<i>Neonotonia wightii</i> (Wight & Arn.) Lackey <sup>i</sup>	Perennial glycine, creeping glycine
<i>Neptunia natans</i> (Willd.) W. Theobald	Not known
<i>Phaseolus vulgaris</i> L.	French bean, kidney bean, navy bean
<i>Pisum sativum</i> L.	Pea, field pea
<i>Securigera varia</i> (L.) Lassen <sup>h</sup>	Crown vetch
<i>Sesbania herbacea</i> (Mill.) R. McVaugh	Not known
<i>Sesbania rostrata</i> Bremek. & Oberm.	Not known
<i>Trifolium alexandrinum</i> L.	Berseem clover, berseem

**Table A1.** (cont'd) Botanical and common names<sup>a</sup> referred to in this review. The first common name listed is the preferred common name.

Botanical name and authority	Common name(s)
<b>Other legumes (cont'd)</b>	
<i>Trifolium ambiguum</i> M. Bieb.	Caucasian clover, kura clover, honey clover
<i>Trifolium dubium</i> Sibth.	Suckling clover
<i>Trifolium fragiferum</i> L.	Strawberry clover
<i>Trifolium glomeratum</i> L.	Cluster clover, ball clover
<i>Trifolium hybridum</i> L.	Alsike clover
<i>Trifolium incarnatum</i> L.	Crimson clover
<i>Trifolium pratense</i> L.	Red clover, cowgrass
<i>Trifolium repens</i> L.	White clover
<i>Trifolium subterraneum</i> L.	Subterranean clover
<i>Vigna umbellata</i> (Thunb.) Ohwi & H. Ohashi	Ricebean
<i>Vigna unguiculata</i> (L.) Walp.	Cowpea
<b>Non-legumes</b>	
<i>Allium cepa</i> L.	Onion
<i>Arctotheca calendula</i> (L.) Levyns	Capeweed
<i>Cocos nucifera</i> L.	Coconut
<i>Eucalyptus globulus</i> Labill.	Tasmanian blue gum
<i>Eucalyptus nitens</i> (Deane & Maiden) Maiden	Shining gum
<i>Lactuca sativa</i> L.	Lettuce
<i>Olax phyllanthi</i> R. Br.	Mistletoe
<i>Parasponia andersonii</i> Planch.	Not known
<i>Sorghum bicolor</i> (L.) Moench s. lat.	Sorghum, sweet sorghum, broom millet
<i>Theobroma cacao</i> L.	Cocoa
<i>Zea mays</i> L.	Maize, corn

<sup>a</sup> Authorities and common names taken from Hartley (1979), National Academy of Sciences 1979, Leigh et al. 1981, Simmons (1981, 1988), Brooker and Kleinig (1990), Wiersema et al. (1990), Tame (1992), Lazarides and Hince 1993, Searle (1996), Boxshall and Jenkyn (2001a, 2001b, 2001c, 2001d), Orchard and Wilson (2001a, 2001b), ILDIS 2002, IPNI 2002, Australian Seed Co. (2003) and Kutsche and Lay (2003). Sometimes ILDIS (2002) gives more than one authority for a particular plant species; in such cases, we give the most recent. 'Not known' refers to species for which none of the above gives a common name.

<sup>b</sup> *Acacia albida* is obsolete; the species is now known as *Faidherbia albida*.

<sup>c</sup> *Acacia arabica* is obsolete; the species is now known as *A. nilotica* subsp. *nilotica*.

<sup>d</sup> *Acacia cyanophylla* is obsolete; the species is now known as *A. saligna*.

<sup>e</sup> *Acacia raddiana* is obsolete; the species is now known as *A. tortilis* subsp. *raddiana*.

<sup>f</sup> *Acacia smallii* is obsolete; the species is now known as *A. farnesiana* var. *farnesiana*.

<sup>g</sup> *Senna siamea* and *S. spectabilis* were formerly *Cassia siamea* and *C. spectabilis*, respectively, now obsolete.

<sup>h</sup> *Coronilla varia* is obsolete; the species is now known as *Securiga varia*.

<sup>i</sup> *Glycine wightii* is obsolete; the species is now known as *Neonotonia wightii*.