


Volume 41 Number 3, 2006

ISSN 0334-5114

# SYMBIOSIS



Balaban Publishers   
In collaboration with the  
International Symbiosis Society  
<http://people.bu.edu/iss/>

Symbiosis Vol. 41, No. 3 (2006)

Cover photo: A young nodule of an adventitious root system established in a decaying nurse log located on the forest floor of a subtropical rainforest. Both Adventitious root system and nurse log are derived from the tree legume *Acacia koa*, which is endemic to the Hawaiian Islands. (Courtesy of James Leary)

© Balaban Publishers

Abstracted in *Biological Abstract*, *CABI*, *Chemical Abstracts*, *Current Contents* and *Current Awareness in Biological Sciences*.

Review article.

## Symbiotic diversity in the cosmopolitan genus *Acacia*

James K. Leary<sup>1</sup>, Paul W. Singleton<sup>2</sup>, Paul G. Scowcroft<sup>3</sup>, and Dulal Borthakur<sup>1\*</sup>

<sup>1</sup>Department Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, 1955 East-West Road, Agricultural Science 218, Honolulu, HI 96822, Tel. +1-808-956-6600, Fax. +1-808-956-3542, Email. dulal@hawaii.edu;

<sup>2</sup>Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, Honolulu, HI 96822;

<sup>3</sup>Institute of Pacific Islands Forestry, USDA Forest Service, Hilo, HI 96720, USA

(Received May 16, 2006; Accepted June 24, 2006)

### Abstract

*Acacia* is the second largest genus within the Leguminosae, with 1352 species identified. This genus is now known to be polyphyletic and the international scientific community will presumably split *Acacia* into five new genera. This review examines the diversity of biological nitrogen fixation symbiosis within *Acacia* as a single genus. Due to its global importance, an extensive body of scientific literature is available on *Acacia*, particularly in the area of symbiotic nitrogen fixation. Most significantly, *Acacia* can be separated into nodulating and non-nodulating species. The nodulating *Acacia* species are symbiotically compatible with a wide range of soil rhizobia. Four different genera of rhizobia are known to nodulate with species of *Acacia*, while most other legumes nodulate with only a single species of rhizobia. The diversity of symbiotic interactions in *Acacia* also corresponds to a wide range of flavonoid inducers produced by the different species. Corresponding to the chemical inducers produced by the host *Acacia*, the nodulating rhizobia also produce a wide range of lipo-chito-oligosaccharide inducers, called Nod factors. The symbiotic diversity we describe for *Acacia* helps to explain the adaptability of different species within this genus to a wide range of extreme environments.

**Keywords:** *Acacia*, subg. *Acacia*, subg. *Aculeiferum*, subg. *Phyllodineae*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Sinorhizobium*, Flavonoid, Nod factor, taxonomy

### 1. Introduction

Leguminosae, with close to 20,000 species, is the third largest family in the plant kingdom (Doyle and Luckow, 2003). Legumes are important to many agronomic systems due to their symbiotic capacity for biological nitrogen fixation (Vance and Graham, 2003). Symbiosis is an interaction between the host legume root system and specialized soil bacteria, collectively referred to as rhizobia, resulting in the morphogenesis of nodules that harbor these nitrogen-fixing bacteria. There are six main genera of rhizobia, including *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*. Recent reports have expanded the list of nodulating symbiotic bacteria to include strains of *Blastobacter*, *Burkholderia*, *Devosia*, *Ensifer*, *Methylobacterium*, *Ochrobactrum*, and *Ralstonia* (Ngom et

al., 2004; Sawada et al., 2003). Much of our current understanding of legume-rhizobia symbiosis has resulted from coordinated research efforts on model systems using the legumes *Glycine max*, *Medicago truncatulata*, and *Lotus japonicus*, along with their rhizobial symbionts (see Choi et al., 2004; Stacey et al., 2004; Young et al., 2003). These legumes have been selected either because of their agronomic importance or due to the practicalities associated with a relatively simple genome that is suitable for genetic manipulation. There are no model systems for woody legumes; however, a substantial amount of scientific information exists describing symbiotic N<sub>2</sub> fixation in woody legumes. One of the most studied genera of woody legumes is *Acacia*.

The current classification of the genus *Acacia* lists 1352 species, thus making it the second largest genus in the Leguminosae family and the largest of the Mimosioideae subfamily (Maslin et al., 2003). *Acacia* consists of three subgenera, including *Acacia* (161 species), *Aculeiferum* (231 species), and *Phyllodineae* (960 species) (Maslin et al., 2003; Vassal, 1972). *Acacia* is a pantropical genus with

\*The author to whom correspondence should be sent.

an overwhelming majority of the species native to Australia (>940 species), but is also well represented in Africa (>140 species), Asia (>90 species), and the Americas (>180 species). New phylogenetic data support reclassification of these subgenera into separate genera (Bukhari et al., 1999; Chappill and Maslin, 1995; Clarke et al., 2000; Grimes, 1999; Luckow et al., 2003; Maslin, 2003; Miller and Bayer, 2000, 2001, 2003; Pedley, 1986; Robinson and Harris, 2000). A proposal has been accepted by the International Botanical Congress to change the *Acacia* type species from *A. nilotica* (African origin) to *A. penninervis* (Australian origin), which will assist in the subdivision of this genus into five new genera (Orchard and Maslin, 2003). In a similar case, the monotypic genus *Faidherbia* (Africa and Middle East origins) has until recently been classified as an *Acacia* species, but is now fully accepted as being distinct from all other *Acacia* spp. (Maslin and Stirton, 1997). As a consequence, much of the available literature describes this species as *A. albida*. Many *Acacia* species are important for industry, rural development, and conservation (Adjers and Hadi, 1993; Awang and Taylor, 1993; Fagg and Stewart, 1994), while other species have been documented as exotic and invasive weeds with negative impacts on the ecosystem (Brown and Carter, 1998; Holmes and Cowling, 1997; Phillips, 1928; Roux, 1961; Weir et al., 2004; Witkowski, 1991). Due to these many important traits, there are several species that have been the focus of numerous research efforts. This review will present the history of *Acacia* symbiosis research in a way that highlights both the universal functions and biological diversities that exist within this complex genus. The review will be presented with the following subheadings: (i) Nodulation of *Acacia*, (ii) Microsymbionts of *Acacia*, (iii) Flavonoid inducers in *Acacia* symbiosis, (iv) Nod factor signaling in *Acacia* symbiosis, (v) Environmental influences on *Acacia* symbiosis, and (vi) Future perspectives.

## 2. Nodulation of *Acacia*

Nodulation is reported to occur in 90% of the Mimosoideae legumes (Allen and Allen, 1981; Faria et al., 1989). The nodule morphology of *Acacia* species has been described as a perennial, multi-lobed 'Mimosoideae' type with peripheral tannin inclusions, corky parenchyma, multiple apical meristems, and well-developed vascular tissues (Allen and Allen, 1936 and 1981; Spratt, 1919). Nodulation typically occurs along the lateral root systems with uneven distributions of nodule clusters (Allen and Allen, 1936).

The subgenus *Aculeiferum* contains many non-nodulating species (Vassal, 1972). A majority of these non-nodulating species are found within the sections *Monacantha* and *Americanae* and are over-represented within the continents of Africa and America (Table 1a).

Interestingly though, species of non-nodulating *Acacia* still display pre-infection events similar to that of nodulating *Acacia*, which include root hair curling and nod gene induction in rhizobia (Harrier et al., 2000; Shaw et al., 1997). Based on these findings, Shaw et al. (1997) suggests that plant functions in post-infection nodule development are missing in these non-nodulating species.

## 3. Microsymbionts of *Acacia*

A majority of the *Acacia* microsymbionts are classified within the genera *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* (De Lajudie et al., 1998; LaFay and Burdon, 2001; Marsudi et al., 1999; Nick et al., 1999a and b). There is wide diversity with respect to their host range, symbiotic effectiveness, and geographic origins. Originally, *Rhizobium* and *Bradyrhizobium* were characterized as fast- and slow-growing rhizobia, respectively. Dreyfus and Dommergues (1981) noted that some *Acacia* spp. nodulated only with fast-growing rhizobia, while other *Acacia* spp. nodulated only with slow-growing (cowpea) rhizobia. They also discovered in rare occurrences, that some species were nodulating with both fast- and slow-growing rhizobia. Since then, more research has identified other species that can nodulate with both fast- and slow-growing rhizobia as well (Table 1b). In most cases, *Acacia* spp. that nodulate with both fast- and slow-growing rhizobia, can only develop an effective symbiosis with one type of symbiont (Assefa and Kleiner, 1998; Frioni et al., 1998; Marsudi et al., 1999; Njiti and Galiana, 1996; Rasanen et al., 2001). Variations in effectiveness can be found even among strains within a single genus of bacteria (Galiana et al., 1990; Murray et al., 2001; Thrall et al., 2000; Turk and Keyser, 1992; Woldemeskel and Sinclair, 1998). Thus, many *Acacia* spp. lack symbiotic specificity for nodulation, but appear to have specificity for nitrogen fixation.

Zhang et al. (1991) first described the *Acacia-Prosopis-Leuceana* nodulation group from a collection of fast-growing rhizobia, isolated from *A. senegal* and *Prosopis chilensis*, growing in Sudan. Rhizobia of this *Acacia-Prosopis-Leuceana* group have the ability to nodulate all three of these legumes as determined by cross-inoculation experiments. Since then, representative strains from this group have been further distinguished from other rhizobia based on phenotypic, biochemical and genomic properties (Haukka and Lindström, 1994; Lindström and Zharan, 1993; Zhang et al., 1991). These representative strains are now classified as *Sinorhizobium arboris* and *Sinorhizobium kostense* (Nick et al., 1999a and b). Lajudie et al. (1994) classified two species, *S. terangaie* and *S. sahelii* from bacterial strains isolated from *A. horrida*, *A. laeta*, *A. mollissima*, *A. raddiana*, and *A. senegal*. In this same study, another group of strains, identified as cluster U, is now classified as *Mesorhizobium plurifarum* (Lajudie et

Table 1. A listing of *Acacia* spp. that have been reported as (A) non-nodulating or (B) nodulating with both fast and slow-growing bacteria.

Species	Subgenus	Origin	Reference
<b>(A) Non-nodulating <i>Acacia</i></b>			
<i>A. adenocalyx</i>	<i>Aculeiferum</i>	Africa	Corby, 1974
<i>A. ataxacantha</i>	<i>Aculeiferum</i>	Africa	Harrier et al., 1997
<i>A. brevispica</i>	<i>Aculeiferum</i>	Africa	Odee and Sprent, 1992
<i>A. excelsa</i>	<i>Aculeiferum</i>	Africa	Beadle, 1964
<i>A. glomerosa</i>	<i>Aculeiferum</i>	America	Faria and Lima, 1998
<i>A. greggii</i>	<i>Aculeiferum</i>	America	Eskew and Ting, 1978
<i>A. loderi</i>	<i>Phyllodineae</i>	Australia	Beadle, 1964
<i>A. macrostachya</i>	<i>Aculeiferum</i>	Africa	Harrier et al., 1997
<i>A. polyphylla</i>	<i>Aculeiferum</i>	America	Souza et al., 1994; Faria and Lima, 1998
<i>A. peridifolia</i>	<i>Aculeiferum</i>	America	Faria and Lima, 1998
<i>A. paniculata</i>	<i>Aculeiferum</i>	America	Faria and Lima, 1998
<i>A. riparia</i>	<i>Aculeiferum</i>	America	Faria and Lima, 1998
<i>A. sakalava</i>	<i>Aculeiferum</i>	Africa	Harrier et al., 2000
<i>A. schweinfurthii</i>	<i>Aculeiferum</i>	Africa	Halliday, 1984
<i>A. stenophylla</i>	<i>Phyllodineae</i>	Australia	Beadle, 1964
<i>A. tucumanensis</i>	<i>Aculeiferum</i>	America	Faria and Lima, 1998
<i>A. velutina</i>	<i>Aculeiferum</i>	America	Faria and Lima, 1998
<b>(B) <i>Acacia</i> nodulating with both fast- and slow-growing symbiotic rhizobia</b>			
<i>A. abyssinica</i>	<i>Acacia</i>	Africa	Assefa and Kleiner, 1998
<i>A. albida</i>	<i>Faidherbia</i>	Africa	Mohamed et al., 2000; Njiti and Galiana, 1996
<i>A. bivenosa</i>	<i>Phyllodineae</i>	Australia	Dreyfus and Dommergues, 1981
<i>A. caven</i>	<i>Acacia</i>	America	Frioni et al., 1998
<i>A. cyclops</i>	<i>Phyllodineae</i>	Australia	Mohamed et al., 2000
<i>A. dealbata</i>	<i>Phyllodineae</i>	Australia	LaFay and Burdon, 2001
<i>A. etbaica</i>	<i>Acacia</i>	Africa	Assefa and Kleiner, 1998
<i>A. farnesiana</i>	<i>Acacia</i>	America	Dreyfus and Dommergues, 1981
<i>A. holosericea</i>	<i>Phyllodineae</i>	Australia	Njiti and Galiana, 1996
<i>A. longifolia</i>	<i>Phyllodineae</i>	Australia	Barnet et al., 1985
<i>A. mearnsii</i>	<i>Phyllodineae</i>	Australia	LaFay and Burdon, 2001
<i>A. melanoxydon</i>	<i>Phyllodineae</i>	Australia	LaFay and Burdon, 2001
<i>A. negrii</i>	<i>Acacia</i>	Africa	Assefa and Kleiner, 1998
<i>A. nilotica</i>	<i>Acacia</i>	Africa	Assefa and Kleiner, 1998; Njiti and Galiana, 1996
<i>A. parramattensis</i>	<i>Phyllodineae</i>	Australia	Barnet and Catt, 1991
<i>A. prasinata</i>	<i>Acacia</i>	Africa	Assefa and Kleiner, 1998
<i>A. polyacantha</i>	<i>Aculeiferum</i>	India	Njiti and Galiana, 1996
<i>A. saligna</i>	<i>Phyllodineae</i>	Australia	Marsudi et al., 1999; Mohamed et al., 2000
<i>A. senegal</i>	<i>Aculeiferum</i>	Africa	Njiti and Galiana, 1996
<i>A. seyal</i>	<i>Acacia</i>	Africa	Assefa and Kleiner, 1998; Dreyfus and Dommergues, 1981
<i>A. sieberiana</i>	<i>Acacia</i>	Africa	Dreyfus and Dommergues, 1981
<i>A. suaveolens</i>	<i>Phyllodineae</i>	Australia	Barnet et al., 1985
<i>A. tortilis</i>	<i>Acacia</i>	Africa	Assefa and Kleiner, 1998
<i>A. tumida</i>	<i>Phyllodineae</i>	Australia	Dreyfus and Dommergues, 1981
<i>A. victoriae</i>	<i>Phyllodineae</i>	Australia	Barnet and Catt, 1991

al., 1998). Recently, Salif et al. (2002) identified new strains of *Sinorhizobium* and *Mesorhizobium* from *A. tortilis* and categorized them in the *Acacia-Prosopis-Leuceana* nodulation group. These studies with *Acacia* introduced several new species of symbiotic bacteria that are all indigenous to Africa. However, many African strains of *Sinorhizobium* can also effectively nodulate many *Acacia* spp. that are native to America (Haukka et al., 1998; Rasanen et al., 2001). Indigenous strains of *Sinorhizobium* and *Mesorhizobium* have since been isolated from some of these American *Acacia* spp. *S. americanus* is one such

strain that has been isolated from several species, and also can nodulate with *L. leucocephala*, which suggests that the American strains of *Sinorhizobium* are related to the *Acacia-Prosopis-Leuceana* nodulation group (Toledo et al., 2003). Although, the rhizobial genes for nodulation and nitrogen fixation (*nodA* and *nifH*) have been found to be variable between *Sinorhizobium* strains from Africa and America, these genes are identical among the geographically diverse strains of *Mesorhizobium* (Haukka et al., 1998; Rasanen et al., 2001).

Most *Acacia* spp. from Australia nodulate with slow-

growing *Bradyrhizobium* (Dreyfus and Dommergues, 1981; LaFay and Burdon, 1998; Marsudi et al., 1999; Räsänen et al., 2001). In Australia, *Bradyrhizobium* is the dominant microsymbiont among *Acacia* spp. (LaFay and Burdon, 2001). However, *Rhizobium* has also been isolated from other Australian species including; *A. glaucoarpa*, *A. mearnsii*, *A. melanoxylon* and *A. saligna*, though *Bradyrhizobium* is still the dominant microsymbiont among these hosts (LaFay and Burdon, 2001; Marsudi et al., 1999). Despite some *Acacia* spp. being able to nodulate with a wide range of rhizobia, the literature suggests that a general delineation exists between the subg. *Phyllodineae*, which nodulates with *Bradyrhizobium*, and the subg. *Acacia* and *Aculeiferum*, which nodulate with *Mesorhizobium* and *Sinorhizobium*. In several phylogenetic studies, the subg. *Phyllodineae* shows a close relationship to the tribe Ingeae, while the subg. *Acacia* shows a close relationship with the tribe Mimoseae (Miller and Bayer, 2001; Miller et al., 2003; Robinson and Harris, 2000). Incidentally, several members of the Ingeae tribe (i.e. *Albizzia* spp.) are known to nodulate with *Bradyrhizobium*, while the members of Mimoseae tribe (i.e. *Prosopis* spp. and *Leuceana* spp.) nodulate with *Mesorhizobium* and *Sinorhizobium* (Doignon-Boucier et al., 1999; Haukka and Lindström, 1994; Lindström and Zharan, 1993; Moreira et al., 1998; Zhang et al., 1991). Thus, there appears to be a congruency between symbiotic compatibility and phylogenetic relationship among the subgenera.

#### 4. Flavonoid Inducers in *Acacia* Symbiosis

Flavonoids, derived from the phenylpropanoid pathway of legumes, are the initial inducers of symbiotic nodulation (Firmin et al., 1986; Kosslak et al., 1987; Peters et al., 1986). Flavonoid profiles vary among different species of *Acacia*, particularly between species that nodulate with *Sinorhizobium* and *Mesorhizobium*, and species that nodulate with *Bradyrhizobium*. For example, the flavonoids apigenin, butein, luteolin, kaempferol, and naringenin induce high *nod* gene expression for *Sinorhizobium* and *Mesorhizobium* strains that nodulate *A. nilotica*, *A. senegal*, *A. seyal*, and *A. tortilis*, while the isoflavonoids diadzein and genistein show little to no induction (Lortet et al., 1996; Salif et al., 2002) (Figs. 1a and 1b). These same isoflavonoids induce Nod factor synthesis in *Bradyrhizobium*, isolated from *A. albida* (syn. *Faidherbia albida*) (Ferro et al., 2000). Interestingly, Shaw et al. (1997) demonstrated that extracts from non-nodulating *A. gregii* and *A. brevispica*, both of the subg. *Aculeiferum*, could also induce *nod* gene expression, suggesting that chemical inducers are present in these species. However, it has yet to be determined whether these inducers are flavonoids or isoflavonoids.

It is well understood that legume symbiosis begins with the root exudation of flavonoids. However, flavonoid

inducers have also been identified in the leaves, phyllodes and seedpods of many *Acacia* spp. (Baraket et al., 1999; Hassan et al., 1973; Lortet et al., 1996; Muhaisan et al., 2002; Novák et al., 2002; Peters et al., 1986; Salif et al., 2002). In fact, Shaw et al. (1997) determined that shoot extracts of *Acacia* actually induced nod gene expression to a higher percentage of a rhizobial test population than root extracts (i.e. shoot 50.5% vs. root 24.4%). Therefore, it is possible that flavonoids present in the litterfall of *Acacia* may have a significant role in enhancing symbiotic interactions. We have observed within the mesic forests of Hawaii, that nodulation of *A. koa* typically occurs at the soil surface under heavy phyllode accumulations. We have also observed hyper-nodulation of adventitious root systems of *A. koa* within decomposing heartwood and phyllode accumulations in the tree canopy (Leary et al., 2004). Thus, even the heartwoods of *Acacia* spp. may have flavonoid symbiotic inducers that are yet to be identified and may be distinguishable between the subgenera.

#### 5. Nod Factor Signaling in *Acacia* Symbiosis

Nod factors are lipo-chito-oligosaccharide (LCO) signals secreted by the symbiotic bacteria in response to flavonoids exuded by the host legume. LCOs trigger root hair curling, infection thread formation and nodule development, which are the initial stages of symbiosis (see reviews by Dénarié et al., 1996 and Spaink, 2000). LCOs consist of a chitin backbone with 3-6 residues of  $\beta$ -1,4-linked N-acetyl-D-glucosamines, along with a N-linked fatty-acid side chain on the non-reducing end of the chitin backbone. Decorations responsible for host specificity are found on the reducing and non-reducing ends of the chitin backbone, which include acetylation, carbamoylation, fucosylation, methylation, methyl-fucosylation, and sulfation. Nod factors have been characterized for strains of *Sinorhizobium*, *Mesorhizobium*, *Rhizobium*, and *Bradyrhizobium* isolated from *A. cyanophylla*, *A. senegal*, *A. tortilis*, and *A. albida* (syn. *Faidherbia albida*), respectively (Ferro et al., 2000; Lortet et al., 1996; Lopez-Lara et al., 1995; Lorquin et al., 1997; Nowak et al., 2004; Salif et al., 2002) (Fig. 2).

Though genetically unrelated, both *Sinorhizobium* teranga bv. *Acaciae* ORS1073 and *Mesorhizobium plurifarimum* ORS1001 (cluster U) have identical major LCOs, and incidentally, both nodulate *A. senegal* (Lorquin et al., 1997). The major LCOs of these two strains are chitopentamers that are O-carbamoylated at the non-reducing end and 6-O-sulfated at the reducing end. Similar LCOs have also been found with other *Sinorhizobium* and *Mesorhizobium* strains, isolated from *A. tortilis* and *A. senegal* (Fig. 2a) (Nowak et al., 2004; Salif et al., 2002). These Nod factors are also chemically similar to the LCOs that are produced by *Rhizobium* sp. strain GRH2, isolated from *A. cyanophylla*, and *R. tropici* strains that are part of

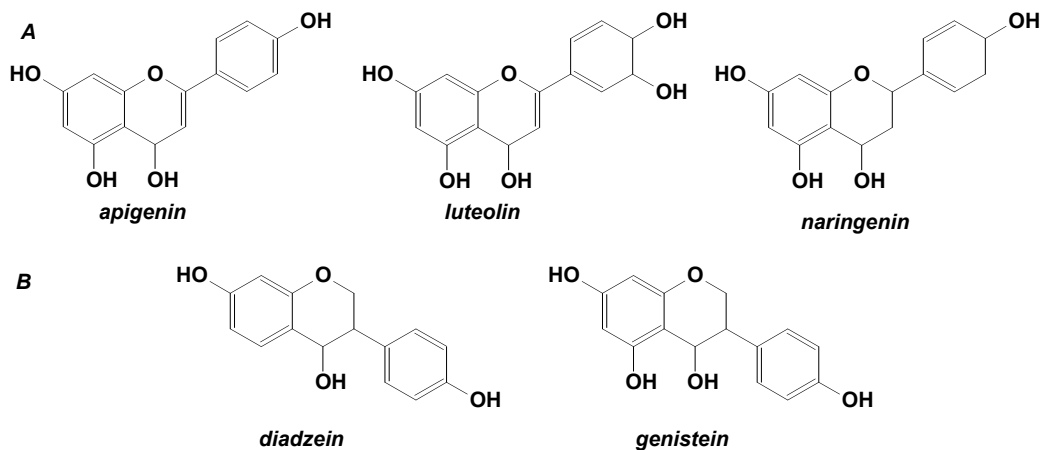


Figure 1. (A) Common flavonoids that induce nod gene function in *Sinorhizobium* and *Mesorhizobium* in association with *Acacia*. (B) Common isoflavonoids that induce nod gene function in *Bradyrhizobium* in symbiosis with *Acacia albida* (syn. *Faidherbia albida*).

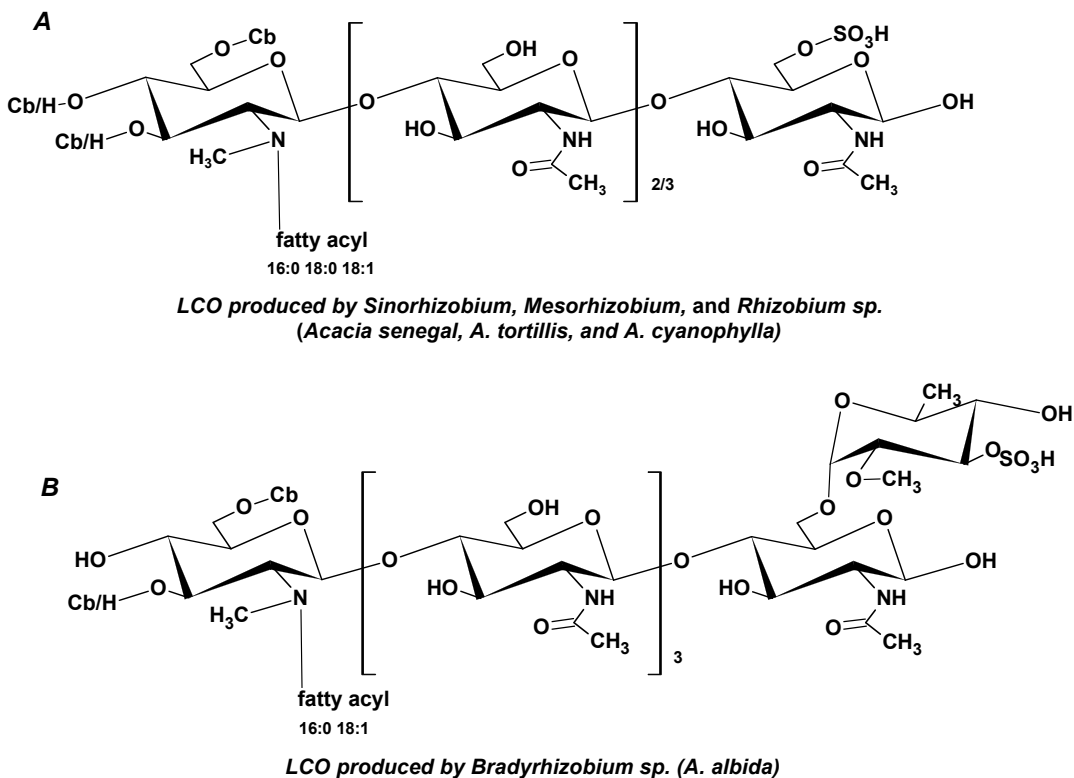


Figure 2. (A) Common Lip-Chito-Oligosaccharides (LCO) produced by *Mesorhizobium*, *Sinorhizobium* and *Rhizobium* sp. (*A. cyanophylla*, *A. senegal*, and *A. tortillis*). (B) Common LCOs produced by *Bradyrhizobium* sp. (*A. albida*) (syn. *Faidherbia albida*). Abbreviations: Cb, (CO-NH<sub>2</sub>) amide group.

the *Acacia-Prosopis-Leucaena* nodulation group (Lopez-Lara et al., 1995; Poupet et al., 1993). Sulfation at the 6-position of the reducing end appears to be a crucial feature for LCOs that confer nodulation within the *Acacia-Prosopis-Leucaena* nodulation group. The sulfated LCOs of *Sinorhizobium teranga* bv. *Acaciae* are easily distinguishable from the non-sulfated Nod factors of

*Sinorhizobium* strains isolated from *Sesbania* (Lortet et al., 1996). In a related study, Folch-Mallol et al. (1996) determined that non-sulfated LCOs produced by a mutagenized *R. tropici* CIAT899 severely reduced nodulation with *Leucaena*, but not with *Phaseolus*. This suggests that 6-O-sulfation at the reducing end of the LCO is responsible for conferring host range compatibility within

the *Acacia-Prosopis-Leucaena* nodulation group. On the other hand, carbamoylation of the non-reducing end of the LCO is not an essential substitution for altering the compatibility with this host range (Lorquin et al., 1997).

Nod factors produced by *Bradyrhizobium* isolates from *A. albida* (syn. *Faidherbia albida*) are different from the Nod factors from *Acacia*-nodulating *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* (Ferro et al., 2000). These LCOs of *Bradyrhizobium* are chitopentamers, with a sulfated 2-O-methyl fucose substitution at the 6-position on the reducing end (Fig. 2b). These *Bradyrhizobium* are unable to nodulate *A. senegal*, as well as other host representatives of the *Acacia-Prosopis-Leucaena* nodulation group. 2-O-methyl-fucosylation at the 6-position is also a common feature of Nod factors derived from *B. japonicum* USDA 110, USDA135, the broad-host range *Rhizobium* sp. NGR 234, and *S. fredii* USDA 257 (Bec-Ferte et al., 1994; Carlson et al., 1993; Sanjuan et al., 1992; Price et al., 1992). Incidentally, NGR234 and USDA257 have been shown to nodulate several Australian *Acacia* spp. within the subgenus *Phyllodineae*, while these strains were unable to nodulate any of the African *Acacia* spp. within the subgenus *Aculeiferum*, which included *A. senegal* (Pueppke and Broughton, 1999). Currently, there are no reports available, which characterize the LCOs of *Bradyrhizobium* species that nodulate Australian *Acacia*. However, it is suspected that *Bradyrhizobium* of Australian *Acacia* will have Nod factors that are similar to the already-characterized Nod factors of other *Bradyrhizobium* strains isolated from *A. albida* (syn. *Faidherbia albida*).

## 6. Environmental Influences on *Acacia* Symbiosis

*Acacia* is a cosmopolitan genus with diverse species adapted to a wide range of extreme environmental conditions. Factors that influence symbiosis of *Acacia* include soil temperature, salinity, and acid pH, (Ashwath et al., 1995; Craig et al., 1991; Deans et al., 1993; Habish, 1970; Habish and Khairi, 1970; Kumar et al., 1999; Lesueur et al., 1993; Lippi et al., 2000; Mohamed et al., 2000; Odee et al., 1995; Surnage et al., 1997; Wilkins, 1967; Zerhari et al., 2000; Zhang et al., 1991). These naturally occurring adaptations make *Acacia* important to agroforestry and land reclamation under extreme environments.

Indigenous strains of symbiotic bacteria, isolated from Australian *Acacia*, were able to survive at higher ambient temperatures than introduced strains (Wilkins, 1967). Several reports have also described African strains, indigenous to hot tropical climates, that survive at temperatures greater than 40°C (Mohamed et al., 2000; Zerhari et al., 2000; Zhang et al., 1991). However, these high temperatures can still have a negative effect on symbiosis; for example, *A. mellifera* is unable to nodulate at temperatures >40°C (Habish, 1970). In Kenya, indigenous

*Acacia* spp. do not nodulate at the hottest times of the year when temperatures can reach 40°C (Odee et al., 1995). In Australia, rates of N<sub>2</sub> fixation for both *A. longifolia* and *A. suaveolens* were highest during the cooler months of the year (Barnett et al., 1985). Thus, the ability of the symbiotic bacteria to survive during the hottest period of the year and still be able to fix nitrogen during the cooler periods may have allowed several of these *Acacia* spp. to become established within the tropical regions of the world.□

*Rhizobium* has been described to have higher salt tolerance than *Bradyrhizobium*, among the strains isolated from *Acacia* (Marsudi et al., 1999; Zou et al., 1995). *Rhizobium* strains isolated from *A. farnesiana* can tolerate NaCl concentrations up to 5% (Surnage et al., 1997). In Sudan, symbiotic rhizobia can grow normally in 3% NaCl and have a higher tolerance than the host *Acacia* (Zhang et al., 1991). Thus, nodulation of *Acacia* requires a much lower salt concentration than the tolerance levels displayed by the rhizobia. Furthermore, salt tolerances vary among different species of *Acacia*. In a controlled inoculation experiment using the same rhizobial strain, *A. catechu* could nodulate at 150 mM NaCl, while *A. nilotica* could only nodulate at 100 mM (Kumar et al., 1999). Intra-species variations in salt tolerance have also been observed in *Acacia* spp. Nodulation of *A. redolens* under saline conditions was found to vary among the provenances with different levels of salt tolerance (Craig et al., 1991). In another case, inoculation with a salt-tolerant *Rhizobium* could improve the salt tolerance of *A. ampliceps* (Zou et al., 1995). Levels of salt tolerance are variable for both *Acacia* and the symbiont; therefore, selection for high salt tolerance of both is an appropriate strategy for forestry establishment in saline environments.

In the humid tropics, soil weathering and acidity are significant impediments to plant growth and productivity. *Bradyrhizobium* is more tolerant to soil acidity than the other genera that nodulate with *Acacia* (Marsudi et al., 1999). *Bradyrhizobium* isolated from *A. mangium* and *A. albida* (syn. *Faidherbia albida*) were found to grow best at pH 4.5 and were tolerant to 100 μM Al (Lesueur et al., 1993). Furthermore, these *Acacia* spp. could also nodulate at the same Al concentration, but only when inoculated with these acid-tolerant strains. Among 36 Australian *Acacia* species, Ashwath et al. (1995) determined that symbiotic N<sub>2</sub> fixation was more sensitive to soil acidity than plant growth *per se*. Thus, the symbionts may be the limiting partners under conditions of acidity and high Al concentrations.

## 7. Future Perspectives

The symbiotic diversity of *Acacia*, described in this review, reflects the overall complexity of this large genus. Once the proposed breakup of this genus is complete, more

research will still be necessary for infra-generic placement of several of these species (Maslin et al., 2003; Miller and Bayer, 2003; Miller et al., 2003; Murphy et al., 2003). Sprent (2001) has suggested that nodulation could serve as a useful key in legume taxonomy. Among the three *Acacia* subgenera, there is an apparent distinction between the Australian species and the African and American species (Maslin et al., 2003). A majority of these Australian *Acacia* nodulate with *Bradyrhizobium*, while a majority of the African and American *Acacia* nodulate with either *Mesorhizobium* or *Sinorhizobium*. Pueppke and Broughton (1999) were able to distinguish between the subg. *Phyllodineae* and subg. *Acacia* and *Acueliferum*, based on nodulation with the broad-host strains NGR 234 and USDA 257. Incidentally, these type strains were also able to nodulate the test species in the tribe Ingeae (related to subg. *Phyllodineae*), but not with the test species in the tribe *Mimoseae* (related to subg. *Acacia*). This supports the use of cross-nodulation tests to determine symbiotic compatibility as a useful taxonomic marker. Expanding these efforts to include other type strains for *R. tropici*, *B. japonicum*, and the *Acacia* type strains for *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* will greatly enhance our understanding of the diversity that exists within the current *Acacia* genus and may also help to verify any new classifications. *Bradyrhizobium* type strains associated with the largest subgenus *Phyllodineae* still need to be identified and used for cross nodulation with *Acacia* spp. Distinct flavonoid and Nod factor combinations, described in this review, can delineate symbiotic diversity among different groups of *Acacia* species. In earlier research, flavonoid and condensed tannin profiles from heartwood, bark and phyllodes were used as chemical markers for classification of *Acacia* (Tindale and Roux, 1969 and 1974; Clark-Lewis and Porter, 1972). Similarly, continuing to distinguish symbiotic inducer flavonoids among *Acacia* spp. would also contribute to new classifications within *Acacia*. The unique quality of certain *Acacia* spp. to nodulate with both fast- and slow-growing rhizobia also deserves further research to elucidate the molecular and physiological mechanisms of these complex interactions. Evidence of bioactive flavonoids present in leaves and phyllodes may also have a significant role in maintaining symbiosis in *Acacia*. Future research may identify unique flavonoids and other phenolics in the litterfall of *Acacia* that promote symbiosis. Based on size, distribution, and utility, *Acacia* may be the most important genus of woody legumes. New research on *Acacia* symbiosis should result in a more global perspective on the role of biological nitrogen fixation in woody legumes.

#### Acknowledgments

This research was supported by USDA-TSTAR grant awards 2004-34135-15174 and 2005-04651. The authors

wish to acknowledge the reviewers for their helpful suggestions.

#### REFERENCES

- Adjers, G. and Hadi, T.S. 1993. *Acacias* in agroforestry. In: *Acacias for Rural, Industrial and Environmental Development*. Awang, K. and Taylor, D.A., eds. FAO Forestry Research Programme for Asia and the Pacific, Bangkok, Thailand, pp. 134–143.
- Allen, O.N. and Allen, E.K. 1936. Root nodule bacteria of some tropical leguminous plants: I. Cross-inoculation studies with *Vigna sinensis* L. *Soil Science* **42**: 61–77.
- Allen, O.N. and Allen, E.K. 1981. *The Leguminosae. A Source Book of Characteristics, Uses and Nodulation*. University of Wisconsin Press, Madison, WI, 812 pp.
- Ashwath, N., Dart, P.J., Edwards, D.G., and Khanna, P.K. 1995. Tolerance of Australian tropical and subtropical *Acacias* to acid soil. *Plant and Soil* **171**: 83–87.
- Assefa, F. and Kleiner, D. 1998. Nodulation pattern and acetylene reduction (nitrogen fixation) activity of some highland and lowland *Acacia* species of Ethiopia. *Biology and Fertility of Soils* **27**: 60–64.
- Awang, K. and Taylor, D. 1993. *Acacia mangium*: growing and utilization. Jointly published with the FAO Forestry Research Support Programme for Asia and the Pacific and Forest Tree Improvement Project, Bangkok, Thailand, 280 pp.
- Baraket, H.H., Souleman, A.M., Hussein, S.A.M., Ibrahim, O.A., and Nawwar, M.A.M. 1999. Flavonoid galloyl glucosides from the pods of *Acacia farnesiana*. *Phytochemistry* **51**: 139–142.
- Barnet, Y.M. and Catt, P.C. 1991. Distribution and characteristics of root-nodule bacteria isolated from Australian *Acacia* spp. *Plant and Soil* **135**: 109–120.
- Barnet, Y.M., Catt, P.C., and Hearne, D.H. 1985. Biological nitrogen fixation and root-nodule bacteria (*Rhizobium* sp. and *Bradyrhizobium* sp.) in two rehabilitating sand dune areas planted with *Acacia* spp. *Australian Journal of Botany* **33**: 595–610.
- Beadle, N.C.W. 1964. Nitrogen economy in arid and semi-arid plant communities. III. The symbiotic nitrogen fixing organisms. *Proceedings of the Linnaean Society of New South Wales* **89**: 273–286.
- Bec-Ferté, M.P., Krishnan, H.B., Promé, D., Savagnac, A., Pueppke, S.G., and Promé, J.C. 1994. Structures of nodulation factors from the nitrogen-fixing soybean symbiont *Rhizobium fredii* USDA257. *Biochemistry* **33**: 11782–11788.
- Brown, J.R. and Carter, J. 1998. Spatial and temporal patterns of exotic shrub invasion in an Australian tropical grassland. *Landscape Ecology* **13**: 93–102.
- Bukhari, Y.M., Koivu, K., and Tigerstedt, P.M.A. 1999. Phylogenetic analysis of *Acacia* (*Mimosaceae*) as revealed from chloroplast RFLP data. *Theoretical and Applied Genetics* **98**: 291–298.
- Carlson, R.W., Sanjuan, J., Bhatt, U.R., Glushka, J., Spaink, H.P., Wijfies, A.H., van, Brussel, A.A.N., Stokkermans, T.J.W., Peters, N., and Stacey, G. 1993. The structure and biological activities of the lipo-oligo-saccharide nodulation signals produced by type I and type II strains of *Bradyrhizobium japonicum*. *Journal of Biological Chemistry* **268**: 18372–18381.
- Chappill, J.A. and Maslin, B.R. 1995. A phylogenetic assessment of tribe *Acacieae*. In: *Advances in Legume Systematics* 7,

- Phylogeny*. Crisp, M. and Doyle, J.J., eds. Royal Botanic Gardens, Kew, UK, pp. 77–99.
- Choi, H.K., Mun, J.H., Kim, D.J., Zue, H., Baek, J.M., Mudge, J., Roe, B., Ellis, N., Doyle, J., Kiss, G.B., Young, N.D., and Cook, D.R. 2004. Estimating genome conservation between crop and model legume species. *Proceedings of the National Academy of Sciences* **101**: 15289–15294.
- Clark-Lewis, J.W. and Porter, L.J. 1972. Phytochemical survey of the heartwood flavonoids of *Acacia* species from arid zones of Australia. *Australian Journal of Chemistry* **25**: 1943–1955.
- Clarke, H.D., Downi, S.R., and Seigler, D.S. 2000. Implications of chloroplast DNA restriction site variation for systematics of *Acacia* (Fabaceae: Mimosoideae). *Systematic Botany* **25**: 618–632.
- Corby, H.D.L. 1974. Systematic implications of nodulation among Rhodesian legumes. *Kirkia* **9**: 301–330.
- Craig, G.F., Atkins, C.A., and Bell, B.T. 1991. Effect of salinity on growth of four strains of *Rhizobium* and their infectivity and effectiveness on two species of *Acacia*. *Plant and Soil* **133**: 253–262.
- Deans, J.D., Ali, O.M., Lindley, D.K., and Nour, H.O.A. 1993. Rhizobial nodulation of *Acacia* tree species in Sudan: Soil inoculum potential and effects of peat. *Journal of Tropical Forest Science* **6**: 56–64.
- Dénarié, J., Debelle, F., and Promé, J.C. 1996. *Rhizobium* lipochito-oligosaccharide nodulation factors: signaling molecules mediating recognition and morphogenesis. *Annual Review of Biochemistry* **65**: 503–535.
- Doignon-Boucier, F., Abdoulaye, S., Willems, A., Torck, U., Dreyfus, B., Gillis, M., and LaJudie, de P. 1999. Diversity of bradyrhizobia from 27 tropical Leguminosae species native of Senegal. *Systematic and Applied Microbiology* **22**: 647–661.
- Doyle, J.J. and Luckow, M. 2003. The rest of the iceberg: legume diversity and evolution in a phylogenetic context. *Plant Physiology* **131**: 900–910.
- Dreyfus, B.L. and Dommergues, Y.R. 1981. Nodulation of *Acacia* species by fast- and slow-growing tropical strains of *Rhizobium*. *Applied and Environmental Microbiology* **41**: 97–99.
- Eskew, D.L. and Ting, I.P. 1978. Nitrogen fixation by legumes and blue-green algal-lichen crusts in a Colorado desert environment. *American Journal of Botany* **65**: 850–856.
- Fagg, C.W. and Stewart, J.L. 1994. The value of *Acacia* and *Prosopis* in arid and semi-arid environments. *Journal of Arid Environments* **27**: 3–25.
- Faria, S.M., de and Lima, H.C., de. 1998. Additional studies of the nodulation status of legume species in Brazil. *Plant and Soil* **200**: 185–192.
- Faria, S.M., de, Lewis, G.P., Sprent, J.I., and Sutherland, J.M. 1989. Occurrence of nodulation in the Leguminosae. *New Phytologist* **111**: 607–619.
- Ferro, M., Lorquin, J., Ba, S., Sanon, K., Promé, J.C., and Boivin, C. 2000. *Bradyrhizobium* sp. strains that nodulate the leguminous tree *Acacia albida* produce fucosylated and partially sulfated Nod factors. *Applied and Environmental Microbiology* **66**: 5078–5082.
- Firmin, J.L., Wilson, K.E., Rossen, L., and Johnston, A.W.B. 1986. Flavonoid activation of nodulation genes in *Rhizobium* reversed by other compounds present in plants. *Nature* **324**: 90–92.
- Folch-Mallol, J.L., Marroquí, S., Sousa, C., Manyani, H., López-Lara, I.M., van der Drift, K.M., Haverkamp, J., Quinto, C., Gil-Serrano, A., Thomas-Oates, J., Spaink, H.P., and Megías, M. 1996. Characterization of *Rhizobium tropici* CIAT899 nodulation factors: the role of *nodH* and *nodPQ* genes in their sulfation. *Molecular Plant-Microbe Interactions* **9**: 151–163.
- Frioni, L., Malatés, D., Irigoyen, I., and Dodera, R. 1998. Promiscuity for nodulation and effectivity in the N<sub>2</sub>-fixing legume tree *Acacia caven* in Uruguay. *Applied Soil Ecology* **7**: 239–244.
- Galiana, A., Chaumont, J., Diem, H.G., and Dommergues, Y.R. 1990. Nitrogen-fixing potential of *Acacia mangium* and *Acacia auriculiformis* seedlings inoculated with *Bradyrhizobium* and *Rhizobium* spp. *Biology and Fertility of Soils* **9**: 261–267.
- Graham, P.H. and Vance, C.P. 2003. Legumes: Importance and constraints to greater utilization. *Plant Physiology* **131**: 872–877.
- Grimes, J.W. 1999. Inflorescence morphology, heterochrony, and phylogeny in the Mimosoid tribes Ingeae and Acacieae (Leguminosae: Mimosoideae). *Botanical Review* **65**: 317–347.
- Habish, H.A. 1970. Effect of certain soil conditions on nodulation of *Acacia* spp. *Plant and Soil* **33**: 1–6.
- Habish, H.A. and Khairi, S.M. 1970. Nodulation of legumes in the Sudan II. *Rhizobium* strains and cross-inoculations of *Acacia* spp. *Experimental Agriculture* **6**: 171–176.
- Halliday, J. 1984. Register of nodulation reports for leguminous trees and other arboreal genera with nitrogen fixing members. *Nitrogen Fixing Tree Research Reports* **2**: 38–45.
- Harrier, L.A., Whitty, P.W., Sutherland, J.M., and Sprent, J.I. 1997. Phenetic investigation of non-nodulating African species *Acacia* (Leguminosae) using morphological and molecular markers. *Plant Systematics and Evolution* **205**: 27–51.
- Harrier, L.A., Whitty, P.W., Sutherland, J.M., and Sprent, J.I. 2000. Pre-nodulation events in non-nodulating species of African *Acacia*. *African Journal of Ecology* **38**: 8–15.
- Haukka, K. and Lindström, K. 1994. Pulsed-field gel electrophoresis for genotypic comparison of *Rhizobium* bacteria that nodulate leguminous trees. *FEMS Microbiology Letters* **119**: 215–220.
- Haukka, K., Lindström, K., and Young, P. 1996. Diversity of partial 16S ribosomal RNA among and within strains of African rhizobia isolated from *Acacia* and *Prosopis*. *Systematic and Applied Microbiology* **19**: 352–359.
- Haukka, K., Lindström, K., and Young, P. 1998. Three phylogenetic groups of *nodA* and *nifH* genes in *Sinorhizobium* and *Mesorhizobium* isolates from leguminous trees growing in Africa and Latin America. *Applied and Environmental Microbiology* **64**: 419–426.
- Holmes, P.M. and Cowling, R.M. 1997. The effects of invasion by *Acacia saligna* on the guild structure and regeneration capabilities of South African fynbos shrublands. *Journal of Applied Ecology* **34**: 317–332.
- Kosslak, R.M., Joshi, R.S., Bowen, B.A., Paaren, H.E., and Appelbaum, E.R. 1990. Strain-specific inhibition of nod gene induction in *Bradyrhizobium japonicum* by flavonoid compounds. *Applied and Environmental Microbiology* **56**: 1333–1341.
- Kumar, H., Arora, N.K., Kumar, V., and Maheshwari, D.K. 1999. Isolation, characterization and selection of salt tolerant rhizobia nodulating *Acacia catechu* and *Acacia nilotica*. *Symbiosis* **26**: 279–288.
- Lafay, B. and Burdon, J.J. 2001. Small-subunit rRNA genotyping of rhizobia nodulating Australian *Acacia* spp. *Applied and Environmental Microbiology* **67**: 396–402.
- Lafay, B. and Burdon, J.J. 1998. Molecular diversity of rhizobia occurring on native shrubby legumes in Southeastern Australia. *Applied and Environmental Microbiology* **64**: 3989–3997.
- Lajudie, P., de, Willems, A., Pot, B., Dewettinck, D., Maestrojuan, G., Neyra, M., Collins, M., Dreyfus, B.L., Kersters, K., and

- Gillis, M. 1994. Polyphasic taxonomy of rhizobia: amendment of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov., and *Sinorhizobium teranga* sp. nov. *International Journal of Systematic Bacteriology* **44**: 715–733.
- Lajudie, P., de, Willems, A., Nick, G., Moreira, F., Molouba, F., Hoste, B., Torck, U., Neyra, M., Collins, M.D., Lindström, K., Dreyfus, B., and Gillis, M. 1998. Characterization of tropical tree rhizobia and description of *Mesorhizobium plurifarum* sp. nov. *International Journal of Systematic Bacteriology* **48**: 369–382.
- Leary, J.K., Singleton, P.W., and Borthakur, D. 2004. Canopy nodulation of the endemic tree legume *Acacia koa* in the mesic forests of Hawaii. *Ecology* **85**: 3151–3157.
- Lesueur, D., Diem, H.G., Dianda, M., and Leroux, C. 1993. Selection of *Bradyrhizobium* strains and provenances of *Acacia mangium* and *Faidherbia albida*: relationship with their tolerance to acidity and aluminium. *Plant and Soil* **149**: 159–166.
- Lindström, K. and Zahran, H.H. 1993. Lipopolysaccharide patterns in SDS-PAGE of rhizobia infecting leguminous trees. *FEMS Microbiology Letters* **107**: 327–330.
- Lippi, D., Paolis, M.R., de, Mattia, E., di, Grego, S., Pietrosanti, T., and Cacciari, I. 2000. Effect of salinity on growth and starvation-survival of a tropical *Rhizobium* strain. *Biology and Fertility of Soils* **30**: 276–283.
- Lopez-Lara, I.M., Drift, K.M., van der, Brussel, A.A., van, Haverkamp, J., Lugtenberg, B.J., Thomas-Oates, J.E., and Spaink, H.P. 1995. Induction of nodule primordia on *Phaseolus* and *Acacia* by lipo-chitin oligosaccharide nodulation signals from broad-host-range *Rhizobium* strain GRH2. *Plant Molecular Biology* **29**: 465–477.
- Lorquin, J., Lortet, G., Ferro, M., Méar, N., Promé, J.C., and Boivin, C. 1997. *Sinorhizobium teranga* bv. *Acaciae* ORS1073 and *Rhizobium* sp. strain ORS1001, two distantly related *Acacia*-nodulating strains, produce similar Nod factors that are O-carbamoylated, N-methylated, and mainly sulfated. *Journal of Bacteriology* **179**: 3079–3083.
- Lortet, G., Méar, N., Lorquin, J., Dreyfus, B., Lajudie, P., de, Rosenberg, C., and Boivin, C. 1996. Nod factor TLC profiling as a tool to characterize symbiotic specificity of rhizobial strains: application to *Sinorhizobium saheli*, *Sinorhizobium teranga* and *Rhizobium* sp. strains isolated from *Acacia* and *Sesbania*. *Molecular Plant-Microbe Interactions* **9**: 736–747.
- Luckow, M., Miller, J.T., Murphy, D.J., and Livshultz, T. 2003. A phylogenetic analysis of the Mimosoideae (Leguminosae) based on chloroplast DNA sequence data. In: *Advances in Legume Systematics 10, Higher Level Systematics*. Klitgaard, B.B. and Bruneau, A., eds. Royal Botanic Gardens, Kew, UK, pp.197–220.
- Marsudi, N.D.S., Glenn, A.R., and Dilworth, M.J. 1999. Identification and characterization of fast- and slow-growing root nodule bacteria from South-Western Australian soils able to nodulate *Acacia saligna*. *Soil Biology and Biochemistry* **31**: 1229–1238.
- Maslin, B.R., Miller, J.T., and Seigler, D.S. 2003. Overview of the generic status of *Acacia* (Leguminosae: Mimosoideae). *Australian Systematic Botany* **16**: 1–18.
- Maslin, B.R. and Stirton, C.H. 1997. Generic and infrageneric classification in *Acacia* (Leguminosae: Mimosoideae): a list of critical species on which to build a comparative data set. *Bulletin of the International Group for the Study of Mimosoideae* **20**: 22–44.
- Miller, J.T. and Bayer, R.J. 2000. Molecular systematics of the Tribe Acaciae (Leguminosae: Mimosoideae). 1995. In: *Advances in Legume Systematics 9, Phylogeny*. Herendeen, P. and Burneau, A., eds. Royal Botanic Gardens, Kew, UK, pp. 180–200.
- Miller, J.T. and Bayer, R.J. 2001. Molecular phylogenetics of *Acacia* (Fabaceae: Mimosoideae) based on the chloroplast MATK coding sequence and flanking TRNK intron spacer region. *American Journal of Botany* **88**: 697–705.
- Miller, J.T. and Bayer, R.J. 2003. Molecular phylogenetics of *Acacia* subgenera *Acacia* and *Aculeiferum* (Fabaceae: Mimosoideae), based on the chloroplast *matK* coding sequence and flanking *trnK* intron spacer regions. *Australian Systematic Botany* **16**: 27–33.
- Miller, J.T., Andrew, R., and Bayer, R.J. 2003. Molecular phylogenetics of the Australian *Acacias*, *Acacia* subg. *Phyllodineae* (Fabaceae: Mimosoideae) based on chloroplast DNA sequences. *Australian Journal of Botany* **51**: 167–177.
- Moreira, F.M.S., Haukka, K., and Young, J.P.W. 1998. Biodiversity of rhizobia isolated from a wide range of forest legumes in Brazil. *Molecular Ecology* **7**: 889–895.
- Mohamed, S.H., Smouni, A., Neyra, M., Kharchaf, D., and Filali-Maltouf, A. 2000. Phenotypic characteristics of root-nodulating bacteria isolated from *Acacia* spp. grown in Libya. *Plant and Soil* **224**: 171–183.
- Muhaisan, H.M.H., Ilyas, M., Mushfiq, M., Parveen, M., and Basudan, O.A. 2002. Flavonoids from *Acacia tortilis*. *Journal of Chemical Research S*: 276–278.
- Murphy, D.J., Miller, J.T., Bayer, R.J., and Ladiges, P.Y. 2003. Molecular phylogeny of *Acacia* subgenus *Phyllodineae* (Mimosoideae: Leguminosae) based on DNA sequences of the internal transcribed spacer region. *Australian Systematic Botany* **16**: 19–26.
- Murray, B.R., Thrall, P.H., and Woods, M.J. 2001. *Acacia* species and rhizobial interactions: Implications for restoration of native vegetation. *Ecological Management and Restoration* **2**: 213–219.
- Ngom, A., Nakagawa, Y., Sawada, H., Tsukahara, J., Wakabayashi, S., Uchiumi, T., Nuntagij, A., Kotepong, S., Suzuki, A., Higashi, S., and Abe, M. 2004. A novel symbiotic nitrogen-fixing member of the *Ochrobactrum* clade isolated from root nodules of *Acacia mangium*. *Journal of General and Applied Microbiology* **50**: 17–28.
- Nick, G., Lajudie, P., de, Eardly, B.D., Suomalainen, S., Paulin, L., Zhang, X., Gillis, M., and Lindström, K. 1999a. *Sinorhizobium arboris* sp. nov. and *Sinorhizobium kostiense* sp. nov., isolated from leguminous trees in Sudan and Kenya. *Journal of Systematic Bacteriology* **49**: 1359–1368.
- Nick, G., Jussila, M., Hoste, B., Niemi, M., Kaijalainen, S., Lajudie, P., de, Gillis, M., Bruijn, F.J., de, and Lindström, K. 1999b. Rhizobia isolated from root nodules of tropical leguminous trees characterized using DNA-DNA dot-blot hybridisation and rep-PCR genomic fingerprinting. *Systematic and Applied Microbiology* **22**: 287–299.
- Njiti, C.F. and Galiana, A. 1996. Symbiotic properties and *Rhizobium* requirements for effective nodulation of five tropical dry zone *Acacias*. *Agroforestry Systems* **34**: 265–275.
- Novák, K., Chovanec, P., Skrdleta, V., Kropáčová, M., Lisá, L., and Nembcová, M. 2002. Effect of exogenous flavonoids on nodulation of pea (*Pisum sativum* L.). *Journal of Experimental Botany* **53**: 1735–1745.
- Nowak, P., Soupas, L., Thomas-Oates, J., and Lindström, K. 2004. *Acacia senegal* and *Prosopis chilensis* nodulating rhizobia *Sinorhizobium arboris* HAMBI 2361 and *Sinorhizobium kostiense* HAMBI 2362 produce tetra- and pentameric LCOs that are N-methylated, O-6-carbamoylated and partially sulfated. *Carbohydrate Research* **339**: 1061–1067.

- Odee, D.W. and Sprent, J.I. 1992. *Acacia brevispica*: a non-nodulating mimosoid legume? *Soil Biology and Biochemistry* **24**: 717–719.
- Odee, D.W., Sutherland, J.M., Kimiti, J.M., and Sprent, J.I. 1995. Natural rhizobial populations and nodulation status of woody legumes growing in diverse Kenyan conditions. *Plant and Soil* **173**: 211–224.
- Orchard, A.E. and Maslin, B.R. 2003. (1584) Proposal to conserve the name *Acacia* (Leguminosae: Mimosoideae) with a conserved type. *Taxon* **52**: 362–363.
- Pedley, L. 1986. Derivation and dispersal of *Acacia* (Leguminosae), with particular reference to Australia, and the recognition of *Senegalia* and *Racosperma*. *Botanical Journal of the Linnean Society* **92**: 219–254.
- Peters, N.K. and Long, S.R. 1988. Alfalfa root exudates and compounds, which promote or inhibit induction of *Rhizobium meliloti* nodulation genes. *Plant Physiology* **88**: 396–400.
- Phillips, J.F.V. 1928. The behaviour of *Acacia melanoxylon* R. Br. (Tasmanian blackwood) in the Knysna forests. *Transactions of the Royal Society of South Africa* **16**: 31–43.
- Poupet, R., Martinez-Romero, E., and Promé, J.C. 1993. Nodulation factors from *Rhizobium tropici* are sulfated or nonsulfated chitopentasaccharides containing an N-methyl-N-acetylglucosaminyl terminus. *Biochemistry* **32**: 10430–10435.
- Price, N.P.J., Relic, B., Talmont, F., Lewin, A., Promé, D., Pueppke, S.G., Mailliet, F., Dénarié, J., Promé, J.C., and Broughton, W.J. 1992. Broad-host range *Rhizobium* species strain NGR 234 secretes a family of carbamoylated and fucosylated nodulation signals that are O-acetylated or sulphated. *Molecular Microbiology* **6**: 3575–3584.
- Pueppke, S.G. and Broughton, W.J. 1999. *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 share exceptionally broad, nested host ranges. *Molecular Plant-Microbe Interactions* **12**: 293–318.
- Räsänen, L.A., Sprent, J.I., and Lindström, K. 2001. Symbiotic properties of rhizobia isolated from *Acacia senegal* and *Prosopis chilensis* nodules in the Sudan. *Plant and Soil* **235**: 193–210.
- Robinson, J. and Harris, S.A. 2000. A plastid DNA phylogeny of the genus *Acacia* Miller (Acacieae, Leguminosae). *Botanical Journal of the Linnean Society* **132**: 195–222.
- Roux, E.R. 1961. History of the introduction of Australian *Acacias* on the Cape Flats. *South African Journal of Science* **57**: 99–102.
- Salif, B., Willems, A., Lajudie, P., de Roche, P., Jeder, H., Quatrini, P., Neyra, M., Ferro, M., Promé, J.C., Gillis, M., Boivon-Masson, C., and Lorquin, J. 2002. Symbiotic and taxonomic diversity of rhizobia isolated from *Acacia tortilis* subsp. *raddiana* in Africa. *Systematic and Applied Microbiology* **25**: 130–145.
- Sanjuan, J., Carlson, R.W., Spaink, H.P., Bhat, U.R., Barbour, W.M., Glushka, J., and Stacey, G. 1992. A 2-O-methylfucose moiety is present in the lipo-oligosaccharide nodulation signal of *Bradyrhizobium japonicum*. *Proceedings of the National Academy of Sciences, USA* **89**: 8789–8793.
- Sawada, H., Kuykendall, L.D., and Young, J.M. 2003. Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts. *Journal of General and Applied Microbiology* **49**: 155–179.
- Shaw, J.E., Reynolds, T., and Sprent, J.I. 1997. A study of the symbiotic importance and location of *nod* gene inducing compounds in two nodulating and two non-nodulating tropical tree species. *Plant and Soil* **188**: 77–82.
- Souza, A.G., de, Silva, M.F., da, and Moreira, F.W. 1994. Capacidade de nodulação de cem leguminosas da Amazônia. *Acta Amazonica* **24**: 9–18.
- Spaink, H.P. 2000. Root nodulation and infection factors produced by rhizobial bacteria. *Annual Review of Microbiology* **54**: 257–288.
- Spratt, E. 1919. A comparative account of the root nodules of the Leguminosae. *Annals of Botany* **33**: 189–199.
- Sprent, J.I. 2000. Nodulation as a taxonomic tool. In: *Advances in Legume Systematics* 9. Herendeen, P.S. and Bruneau, A., eds. Royal Botanic Gardens, Kew, UK, pp. 21–43.
- Sprent, J.I. and Parsons, R. 2000. Nitrogen fixation in legume and non-legume trees. *Field Crops Research* **65**: 183–196.
- Stacey, G., Vodkin, L., Parrott, W.A., and Shoemaker, R.C. 2004. National Science Foundation-Sponsored Workshop Report. Draft Plan for Soybean Genomics. *Plant Physiology* **135**: 59–70.
- Surnage, S., Wollum, A.G., Kumar N., and Nautiyal, C.S. 1997. Characterization of *Rhizobium* from root nodules of leguminous trees growing in alkaline soils. *Canadian Journal of Microbiology* **43**: 891–894.
- Thrall, P.H., Burdon, J.J., and Woods, M.J. 2000. Variation in the effectiveness of symbiotic associations between native rhizobia and temperate Australian legumes: interactions within and between genera. *Journal of Applied Ecology* **37**: 52–65.
- Tindale, M.D. and Roux, D.G. 1969. A phytochemical survey of the Australian species of *Acacia*. *Phytochemistry* **8**: 1713–1727.
- Tindale, M.D. and Roux, D.G. 1974. An extended phytochemical survey of Australian species of *Acacia*: chemotaxonomic and phylogenetic aspects. *Phytochemistry* **13**: 829–839.
- Toledo, I., Lloret, L., and Martinez-Romero, E. 2003. *Sinorhizobium americanus* sp. nov., a new *Sinorhizobium* species nodulating native *Acacia* spp. in Mexico. *Systematic and Applied Microbiology* **26**: 54–64. Erratum in: *Systematic and Applied Microbiology* **26**: 319.
- Turk, D. and Keyser, H.H. 1992. Rhizobia that nodulate tree legumes: specificity of the host for nodulation and effectiveness. *Canadian Journal of Microbiology* **38**: 451–460.
- Vassal, J. 1972. Apport des recherches ontogéniques et seminologiques à l'étude morphologique, taxonomique et phylogénique du genre *Acacia*. *Bulletin de la Société d'Histoire Naturelle de Toulouse* **108**: 105–247.
- Weir, B.S., Turner, S.J., Park, W.B., Silvester, D.C., and Young, J.M. 2004. Unexpectedly diverse *Mesorhizobium* strains and *Rhizobium leguminosarum* nodulate native legume genera of New Zealand, while introduced legume weeds are nodulated by *Bradyrhizobium* species. *Applied and Environmental Microbiology* **70**: 5980–5987.
- Wilkins, J. 1967. The effects of high temperatures on certain root-nodule bacteria. *Australian Journal of Agricultural Research* **18**: 299–304.
- Witkowski, E.T.F. 1991. Effects of invasive alien *Acacias* on nutrient cycling in the coastal lowlands of the Cape fynbos. *Journal of Applied Ecology* **28**: 1–15.
- Woldemeskel, E. and Sinclair, F.L. 1998. Variations in seedling growth, nodulation and nitrogen fixation of *Acacia nilotica* inoculated with eight rhizobial strains. *Forest Ecology and Management* **104**: 239–247.
- Young, N.D., Mudge, J., and Ellis, T.H. 2003. Legume genomes: more than peas in a pod. *Current Opinion in Plant Biology* **6**: 199–204.
- Zerhari, K., Aurag, J., Khbaya, B., Kharchaf, D., and Filali-Maltouf, A. 2000. Phenotypic characteristics of rhizobia isolates nodulating *Acacia* species in the arid and Saharan regions of Morocco. *Letters in Applied Microbiology* **30**: 351–357.

- Zhang, X., Harper, R., Karsisto, M., and Lindström, K. 1991. Diversity of *Rhizobium* bacteria isolated from the root nodules of leguminous trees. *Journal of Systematic Bacteriology* **38**: 89–98.
- Zou, N., Dart, P.J., and Marcar, N.E. 1995. Interaction of salinity and rhizobial strain on growth and N<sub>2</sub> fixation by *Acacia ampliceps*. *Soil Biology and Biochemistry* **27**: 409–13.