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Nodulation and Nitrogen Fixation in Members of the Rhamnaceae (*Colletia*, *Retanilla*, *Talguenea* and *Trevoa*) Growing in the Chilean Matorral

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Abstract

Five species in four genera of the Rhamnaceae were surveyed for root nodules in the matorral of central Chile. All species in the area possessed root nodules and for one genus, *Retanilla*, and two species, *R. ephedra* and *R. stricta*, this represents the first report of root nodules and nitrogen fixation. Acetylene reduction and $^{15}N_2$ fixation was measured in all species. Nitrogen fixation is shown to be associated with the apical portions of nodules and it is assumed that in these species which are drought deciduous, or otherwise cease functioning in the dry season, annual regrowth of nodule tips provides new active nodule material.

Key words: Nitrogen fixation, Rhamnaceae, *Colletia*, *Retanilla*, *Talguenea*, *Trevoa*, matorral, nodulation.

1. Introduction

The family Rhamnaceae typically colonises Mediterranean climate areas of the Americans and the Pacific. Many species show abilities to survive long dry summers and are important members of the chaparral of North America and the matorral of South America.

Two tribes within the family contain known nodulated members, *Ceanothus* (tribe Rhamneae) is a widespread northern hemisphere shrub genus of 55 species in which 31 species have been reported to bear root nodules (Bond, 1983). Nitrogen fixation has been reported in several genera and their importance as forest underbrush has been studied (Youngberg and Wollum, 1976).

The other tribe, Colletieae, contains a diverse range of species centered in South America but also occurring in New Zealand and Australia. Five genera in this group have been reported nodulated although in some cases these reports

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consist only of verbal communications. *Discaria*, found in South America, New Zealand and Australia, was first recorded as nodulated in 1958 (Morrison and Harris) and later reported capable of $^{15}N_2$ fixation (Morrison, 1961). Five of the ten known species are currently reported as nodulated. *Colletia* was first reported nodulated in 1976 (Bond, 1976) on the basis of specimens from the Glasgow Botanic Gardens. These observations have been widened to field material (Medan and Tortosa, 1976) and to other Botanic Gardens (Bond and Becking, 1982) and three of the seventeen known species are now reported nodulated. One species of *Kentrothamnus* was reported to be nodulated by Medan and Tortosa (1981) while a verbal report from Dr. Kummerow to Dr. Bond (Bond, 1983) indicated that *Talguenea* is also nodulated. Finally, two out of six species of *Trevoa* have been shown to be nodulated (Rundel and Neel, 1978; Medan and Tortosa, 1981).

The southern part of South America is the centre of distribution of Colletieae, if not for the Rhamnaceae as a whole. In addition to the five mentioned above the tribe contains two other genera, *Adolphia* and *Retanilla*, and it has been postulated (Akkermans, 1979; Bond and Becking, 1982) that all members in the tribe are descended from a common nodulated ancestor.

In the mid-elevation matorral of the coastal ranges of central Chile various plant associations often include up to five species of Colletieae. This matorral habitat is physiognomically similar to the Southern Californian chaparral and is essentially Mediterranean in climate with dry hot summers and moist cool winters (Hajek and de Castri, 1975; Miller et al., 1977). The vegetation and soils are those of a degraded sclerophyll forest which has been subject to intensive wood cutting and/or cattle grazing over a long period (Armesto and Martinez, 1978). Nitrogen content in soils of the matorral is low and for several species growing there, there is a significant response to added N fertiliser (Kummerow et al., 1982). The present work was designed to test for the presence of nodules and nitrogen fixation in five members of the Colletieae in the matorral vegetation north of Santiago (Table 1).

2. Materials and Methods

Study site

The study site forms part of the central Chilean matorral and is within the Fundo Santa Laura 60 km NNW of Santiago de Chile (lat 33°04′S, long 71°00′W, elevation ca 1000 m). The vegetation of the area is dominated by the evergreen sclerophyll species *Lithraea caustica* (Mol.) H. et Arn. (Anacardiaceae) *Cryptocarya alba* (Mol.) Looser (Lauraceae) and *Quillaja saponaria* Mol. (Rosaceae) and the semi- or drought-species *Colliguaya odorifera* Mol. (Euphorbiacease) *Trevoa trinervis* Miers (Rhamnaceae) and *Satureja gilliesii* (Grah.) Brig. (Labiatae). The vegetation, soils and climate of the area have been described in detail in previous communications (Thrower and Bradbury, 1977; Mooney, 1977; Miller et al., 1980). The work described here was carried out during spring (August) 1983. This period

Table 1. Members of the tribe Colletieae (Rhamnaceae)[1] occurring in the mid-elevation matorral of the coastal ranges and their respective habit and life forms

Species	Habit	Life-form
Talguenea quinquenervia (Gill et Hook)	Spiny shrub or small tree	Drought deciduous
Trevoa trinervis (Miers)	Spiny shrub	Drought deciduous and photosynthetic stems
Retanilla stricta (Hook et Arn)	Spiny shrub	Drought deciduous and photosynthetic stems
Retanilla ephedra (Vent et Brong)	Shrub	Photosynthetic stem
Colletia spinosissima (Gmel)	Spiny shrub	Photosynthetic stem

[1] Nomenclature according to Munoz-Pizarro (1966).

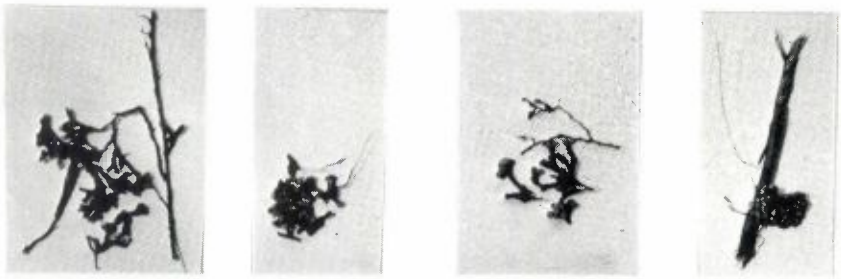
coincides with the rainy season and soils were uniformly moist over the sampling period and soil temperatures averaged 10-12°C. Spring growth in many species was well advanced. By the end of August flower buds were well developed in *Retanilla ephedra* and leaf buds in *R. stricta*, *T. quinquenervia* and *T. trinervis*.

Nodule recovery

The A horizon of the area is poorly developed with up to 18% gravel 40% sand and 30% silt (Kummerow et al., 1982). In some places, plants were growing amongst large boulders and excavation of nodules was made with difficulty, using a long crowbar.

Acetylene reduction

The nodules, with attached roots, were incubated in 100ml bottles, fitted with septum stoppers. Ten percent v/v acetylene was added to the bottles by syringe and they were incubated in the field. After one hour, 7ml sub-samples of the incubation gas phase were added to previously evacuated 7ml blood tubes. On return to the laboratory 0.1ml samples were analysed by gas chromatography for acetylene and ethylene. Calculation of acetylene reduction was made on the basis of acetylene as the internal standard with appropriate corrections for barometric pressure and temperature. All calculations were made on nodule dry weight basis.



Retanilla ephedra *Retanilla stricta* *Colletia spinosissima* *Trevoa trinervis*

Talguenea quinquenervia

cm

Figure 1a: Root nodules of five species of Rhamnaceae excavated at Santa Laura.

Figure 1b: Total nodules recovered from beneath one *Talguenea* plant. Top 25cm of soil was excavated and yielded 1.75g dry weight of nodules.

$^{15}N_2$ *uptake*

Nitrogen gas containing 95 atom% excess ^{15}N was mixed with oxygen to give pO_2 of 0.20. This was diluted with air to give a final ^{15}N enrichment of 30 atom% excess and stored in a flask over water. In the field, nodules were placed in 30ml universal bottles fitted with new septum seals. The bottles were then evacuated three times using a 50ml syringe and 30ml of the $^{15}N_2/O_2$ mixture added to the evacuated universal. After four hours incubation, 3.0ml C_2H_2 was added to stop the uptake of $^{15}N_2$ and acetylene reduction followed over the subsequent 30 minutes. With each set of nodule samples, duplicate blank tubes of $^{15}N_2$ mixture were taken for future analysis of ^{15}N abundance in the gas phase, so that quantitative estimates of N_2 fixation could subsequently be determined. The above techniques are described in greater detail in Silvester (1983).

3. Results

Nodulation

Typical *Frankia* type nodules were found on all five species of Rhamnaceae occurring at Santa Laura (Table 1). On occasions nodules were not found on individual plants but this is attributed to poor excavation rather than absence of nodules in the field. It can now be assumed that nodules normally occur on these five species. However at another site (Cuesta Barriga) ten plants of *Trevoa* which were excavated did not have nodules while four plants of *Talguenea* at Farellones Villa Paulina all possessed nodules and reduced acetylene.

The root nodules are typical corralloid clusters (Fig. 1a) usually occurring, at least at this time of the year, near the main root stock, often lodged against or between rocks, and for some undetermined reason more predominantly on the north side of the plant. Small nodules were seldom seen, although there was some indication that the finer roots were producing new nodules. An excavation of one 3m tall *Talguenea* plant yielded 1.75g dry weight of nodules in the top 25cm of soil (Fig. 1b). Eighty per cent of these nodules were found on the north side and within 40cm of the main root stock.

Acetylene reduction

Rates of acetylene reduction were assayed on several occasions in the field. In all cases root nodules were active, ranging up to 47 nmoles $C_2H_2 mg^{-1}\ h^{-1}$ in a 12 hour incubation of *Talguenea* nodules (Table 2). Very large nodule masses such as *Talguenea* (Table 2) of 600mg showed much lower acetylene reduction then smaller, presumably younger, nodules which is commonly the case in perennial nodules in which the older tissue is relatively inactive. Even under long incubation times with detached nodules, acetylene reduction remained remarkably constant and the rates cover a normal range of activities for nodules.

Table 2. Acetylene reduction activity of root nodules collected and assayed in the field. Each was sampled several times after introduction of C_2H_2 to establish linearity of C_2H_2 reduction.

Species		Nodule weight mg (dry)	C_2H_2 reduction nmoles $mg^{-1}h^{-1}$			
			1.1 h	3.0 h	4.0 h	12 h
R. stricta	(1)	125	23.9	22.3	21.7	
	(2)	50	28.8	28.2	26.7	
	(3)	15				24.9
R. ephedra		180	14.5	12.8	12.5	
C. spinosissima	(1)	50	33.1	30.0	28.4	
	(2)	90	21.4	24.1	22.9	
T. quinquenervia	(1)	600	16.9	15.9	15.1	
	(2)	200	33.8	31.3	29.7	
	(3)	7	34.7			47.0
T. trinervis	(1)	220	19.6	17.1	16.6	10.6
	(2)	260	25.7	22.0	15.0	11.4

$^{15}N_2$ *uptake*

$^{15}N_2$ (30 atom% excess ^{15}N) was applied to nodules in the field and incubated at soil temperature (ca 12°C). The results presented in Table 3, show that all samples and species incorporated significant $^{15}N_2$. All samples were taken within a two hour period on the same day (starting 10.00h). The ratio C_2H_2/N_2 shows considerable variation; both within and between species, which in some cases is due to variable acetylene reduction and in others is due to variable $^{15}N_2$ uptake. No great significance can be attached to the specific ratios, although the mean ratio 3.22 approaches closely the theoretical value of 3.0. It should be recognised that acetylene reductions were conducted for 30 minutes after a four hour $^{15}N_2$ incubation and despite the fact that good activity may be sustained for at least four hours there is also evidence for a decline in nitrogenase activity of some replicates over this time (Table 2).

Site of nitrogen fixation

Nodules of the Rhamnaceae, like those of most actinorhizal plants, consist of elongated perennial lobes up to 20 mm long with apical meristems. To test site of fixation, several nodule lobes of *R. ephedra* and *Colletia* were dissected into apical, middle and basal 4mm sections after exposure to $^{15}N_2$ for four hours. It is apparent (Table 4) that apical sections are both higher in nitrogen and N_2 fixation than basal sections, with intermediate values in the middle sections.

Table 3. $^{15}N_2$ uptake by root nodules. Excised nodules were exposed to $^{15}N_2$ (pN_2 0.80, 30 atom% excess ^{15}N) for four hours, followed by 30 minute C_2H_2 reduction assay.

Species	Nodule wt mg	nmoles C_2H_4 $mg^{-1}h^{-1}$	Atom% excess ^{15}N	nmoles N $mg^{-1}h^{-1}$	$R=\frac{C_2H_2}{N_2}$
R. ephedra	73.4	5.94	0.277	2.73	2.18
	20.6	13.7	0.266	2.53	5.42
Colletia	40.0	3.95	0.164	1.90	2.08
	47.6	4.40	0.185	1.99	2.21
R. stricta	48.4	14.04	0.340	3.72	3.77
	94.1	14.40	0.520	6.33	2.27
	102.6	11.76	0.448	4.77	2.47
Trevoa	31.1	13.4	0.370	5.10	2.63
	20.7	15.3	0.330	3.56	4.30
	44.1	14.7	0.352	4.24	3.47
Talguenea	45.5	9.17	0.406	2.97	3.09
	25.8	11.6	0.350	2.70	4.30
	34.3	12.3	0.445	3.32	3.70
					$\overline{X}$ 3.22
					s=1.04

Table 4. Site of N_2 fixation in nodules. Nodules were incubated in $^{15}N_2$ (pN_2= 0.80, 30 atom% excess $^{15}N_2$) for four hours, divided into apical, middle and basal 4mm sections and separately analysed for ^{15}N.

Species	Position	Weight mg	% total N	Atom% excess ^{15}N	nmole N_2 $mg^{-1}h^{-1}$
R. ephedra	apical	16.7	4.11	0.550	7.16
	middle	14.8	3.53	0.212	2.37
	basal	18.5	2.97	0.090	0.85
Colletia	apical	12.0	4.26	0.242	3.27
	middle	9.0	4.55	0.210	3.05
	basal	13.1	2.83	0.204	0.94

4. Discussion

The number and taxonomic diversity of known actinorhizal plants has increased markedly over the last 30 years. Bond (1983) records that 20 genera and 194 species in eight families are now confirmed as actinorhizal plants. This report adds one further genus *Retanilla*, and two species *R. stricta* and *R. ephedra* to that list and confirms the finding for the three other genera.

The nodulation patterns in the Rhamnaceae have certain similarities with that of the Rosaceae. In the Rosaceae it appears that a high proportion of the Dryadeae are nodulated but only one, *Rubus*, in the Rubeae. Similarly in the Rhamnaceae, *Ceanothus* is the sole member of the tribe reported nodulated while six of the seven genera in the Colletieae are now reported to be nodulated. It is likely that the remaining genus *Adolphia* will also be discovered to be actinorhizal.

All of the Rhamnaceae have the capacity to survive on dry infertile sites, and the Colletieae are uniquely capable of surviving long dry periods. Most of the plants are spinose and either drought deciduous or with photosynthetic stems. Under these conditions the survival and regrowth of perennial nodules poses some interesting physiological problems. It is certain that the large nodules found on some of the species, have survived several dry periods. While the experiments described here show that the apical nodule portions are more active than the proximal portions, it is not certain whether only the recent regrowth of nodules is active or whether activity is regenerated in the older nodule portion. The finding (Table 4) that both total N and ^{15}N enrichment are highest in the apical portion is equivocal in this context and it would be most interesting to discover the mode of annual growth of nodules and the extent of which old tissue is capable of regenerating activity.

The total mass of nodules in such sites as Santa Laura is almost impossible to determine. The limited excavation of one *Talguenea* plant reported here, yielded 1.75g of nodules, while a further excavation of a similar plant yielded 6.1g. Mature *Talguenea* plants, seldom achieve more than 3m in height with a canopy diameter of 3m and numbers ranging up to several hundred per hectare. With this cover of plant material the total nodule mass is unlikely to exceed more than one or two kilograms of nodules per hectare compared with 100-2000 kg ha^{-1} under lupins in sand dunes in New Zealand (Sprent and Silvester, 1973).

The results in the present study were obtained in the early part of the growing season and over a very short period when soil temperatures were still around 10°C. Further work is in progress which will elaborate on the response of nodules to increasing temperature during the growing season and the response to increasing moisture stress later in the season.

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