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Variation with soil depth, topographic position and host species in the capacity of soils from an Australian locale to nodulate *Casuarina* and *Allocasuarina* seedlings

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Abstract

Sandy alluvial soils in a floodplain supporting a native stand of *Casuarina cunninghamiana* Miq. produced about three times as many nodulated seedlings and more than twice as many nodules per nodulated seedling on roots of baited *Casuarina* spp. than did clay loam red earth soils from the adjacent valley slope. Moist and well-aerated subsurficial alluvial sands had the greatest nodulation capacity of all the soils sampled. For all topographic positions, soil samples from depths greater than 20 cm promoted 76% more nodulated Casuarina seedlings than samples from the surficial 20 cm.

Seedlings of three provenances of *C. cunninghamiana*, together with seedlings of *C. glauca* Sieb. ex Spreng., *C. cristata* F. Muell ex Miq. and *C. obesa* Miq. developed significantly more nodules per pot and nodules per nodulated seedling in soils from this locale than seedlings of two *Casuarina equisetifolia* Forst. provenances. Seedlings of two provenances of *Allocasuarina torulosa* (Ait.) L. Johnson had fewer than 1% nodulated seedlings, a significantly lower level by far than that of *Casuarina* seedlings. *A. torulosa* provenances also had significantly fewer nodulated seedlings per pot and nodules per nodulated seedling than all Casuarina hosts excepting one poorly-nodulated provenance of *C. equisetifolia*.

Nodulated seedlings of all *Casuarina* species had the capacity to fix atmospheric N_2, as indicated by acetylene-reduction capability. The presence of yellow cladodes and low rates of acetylene reduction per plant for *C. cristata* Miq. suggest that this association was poorly effective.

Introduction

Nodulation can be scarce or absent in actinorhizal *Casuarina* and *Allocasuarina* species growing within their native ranges in Australia, and even within their native habitats. Hence reservations as to the ecological significance of N_2 fixation by these species have been expressed (Bond, 1976; Lawrie, 1982). Lawrie (1982) examined field nodulation of nine species of *Casuarina* and *Allocasuarina* in Victoria finding nodules on only 15% of 336 plants examined and at only 17% of 77 sites. Baiting studies in a glasshouse confirmed field findings in 88% of the cases. The exceptions were for 7 sites where habitat soil induced nodulation in the glasshouse but where no field nodulation was observed. Lawrie found no nodules at sites more than 70 km from the coast.

Reddell *et al.* (1986) found that 4 of the 5 *Casuarina* species studied in natural populations were regularly nodulated in 88 sites examined. Only 9 out of a total of 17 *Allocasuarina* species examined were found to bear nodules in the field, and then only a minority of the plants examined

had nodules. Low available-phosphorous level in soil was associated with low nodulation, but no other soil chemical properties had any apparent relationship to nodulation. Baiting of field soils under glasshouse conditions with seedlings of Casuarinaceae confirmed patterns of nodulation similar to those observed in the field.

In addition to low soil P and the absence of infective microbial populations, moisture stress (Kant and Narayana, 1978), low soil temperatures (Redell *et al.*, 1985), high levels of available N (Rodriguez-Barrueco, 1972), deficiencies of nutrients other than N and P (Robson and Loneragan, 1978) and low soil pH (Bond, 1957; Coyne, 1973) may inhibit field nodulation by *Frankia* and Rhizobium.

Failure to find nodules in the field might be a result of the location of roots of a *Casuarina* or *Allocasuarina* species in deep soil strata that are difficult to sample. For example, *Allocasuarina decaisneana* (F. Muell.) L. Johnson, which grows in Australia's arid interior, usually has long tap roots with little lateral or fine-root development near the surface of the soil. Few nodulated plants of this species have been reported (Torrey, 1983). Perhaps fine-root proliferation and nodulation occur in this species primarily at depths where permanent soil moisture exists.

Nodulation tends to decrease when species of Casuarinaceae are planted outside their native range or habitat. For example, *C. cunninghamiana* Miq. plantations established within the range of the species by P. Reddell and P. Ryan near Gympie, Queensland did not nodulate in upland soil unless seedlings were inoculated (Personal communication, P. Ryan, Queensland Forestry Commission, Gympie Research Station, Queensland, Australia). Another example is the failure of *C. cunninghamiana* to nodulate in New Zealand unless plants are inoculated. Redell and Bowen (1986) using nodule suspensions found host-endophyte specificity within Casuarinaceae was expressed mainly at the generic level. They found marked cross-inoculation within the genus Casuarina and, to a lesser extent, within the genus Allocasuarina. Few nodule sources produced nodules in species of the other genus.

The extreme variability in nodulation reported in field surveys of Casuarinaceae might be due, in part, to large differences in the nodulation capacity of soils over small distances. The density and nature of the *Frankia* population in a soil might vary greatly with small differences in moisture, aeration, nutrient availability and the proximity and species of host plants. We hypothesized that soil infective capacity would vary with topographic position, soil depth and host species baited even within the confines of a single location along a 1-km stretch of the Murrumbidgee River in the Australian Capital Territory (A.C.T.). We further hypothesized that *Allocasuarina torulosa* (Ait.) L. Johnson would not nodulate as readily as *Casuarina* species in soils from this locale where only *C. cunninghamiana* was present.

Materials and methods

A 1-km section of river bottomland and valley slope along the east bank of the Murrumbidgee River at Casuarina Sands (35° 19′ latitude and 148° 97′ longitude) in the Australian Capital Territory was selected for study. A native stand of mature *Casuarina cunninghamiana* trees occurred on alluvial deposits (Entisol) adjacent to the steep (20 to 70%) slope of an adjacent valley wall.

Soils on slopes were stony red earths (Oxic or Ultic Paleustalfs) and yellow earths (Udic Haplustalfs) (Sleeman and Walker, 1979). Plastic, heavy clay layers commonly occurred under upper slope soils at depths of approximately 60 cm and are interpreted as older, truncated buried soils, or subsolum features originating from a wetter period of greater soil stability (Van Dijk, 1959, 1969). The red and yellow earth soils exhibit weak profile differentiation and test neutral in reaction. They consist of massive porous materials and can grade in texture from loamy near the surface to clay loam at depths of 40 to 60 cm. Lower slope soils are formed in deeper colluvial material. Rocks and rock outcroppings also occur on the steeper valley slopes. These soils are typically low in N, organic matter, and very low in available P (Sleeman and Walker, 1979). Soil pH is generally acid to neutral and salt content is low.

Alluvial deposits consisted of siliceous sands neutral in reaction with, at most, a slightly darkened A1 horizon and an array of sedimentary layers beneath the surface. These soils are typically low in N, P and other nutrients, have a slightly acid

to neutral pH and are low in salt content (Sleeman and Walker, 1979).

Three linear transects were established between randomly-selected points at the river's edge and the nearest adjacent point at the crest of the valley wall. Six soil samples were taken along each transect: at river's edge, in the middle of the alluvial plain, in alluvial sand at the base of the valley slope, and at the base, midslope and crest of the valley slope. Soil samples were taken in October at a time of normal river flow with the river level at 462 m in elevation. The maximum elevation at the crest of the adjacent slope was 560 m.

Soil was sampled at each point in 20-cm depth increments to a depth of 80 cm. A clean shovel was used at each point for each sample depth to minimize contamination from adjacent soils. Soils were mixed with an equal volume of Vermiculite to balance the water holding capacities of the mixtures containing variably-textured soils and 100-ml portions of each sample were placed in 4-cm diameter by 9 cm deep cylindrical pots. Each pot had one hundred and fifty seeds sown from each of 10 different Casuarina and Allocasuarina seed sources. Seeds were from collections made available by the Division of Forest Research of CSIRO in Canberra (Table 1). Additional soil samples from each combination of transect, topographic position and depth were combined, steam sterilized twice for 30 minutes at 121°C and 103 kPa in an autoclave, and seeded as described above to assess the importance of environmental and seed-born *Frankia* contamination in the glasshouse study.

The experiment was conducted in a naturally-illuminated glasshouse with a temperature range of 18 to 37°C (16 h day) and 12 to 22°C (8 h night) and relative humidity ranging from 20 to 100%. The natural light period was extended to 16 h with overhead fluorescent lighting. Pots with slotted bases to allow for drainage were arranged in a random manner with respect to treatments. Plants were watered daily with distilled water applied as a mist to minimize splashing.

Seedlings were harvested eighty days after germination with the number of surviving seedlings, number of seedlings nodulated, number of nodules per nodulated seedling, plant shoot lengths and plant root lengths measured for each pot. Seedlings were removed from the soil mixture under a gentle stream of water to minimize root damage, and those that could not be examined immediately were preserved in 70% ethanol for later measurement.

Two fresh subsamples, each with 10 randomly-selected, nodulated plants per nodulated species or

Table 1. Sources of Casuarina and Allocasuarina seeds

Tree species	CSIRO Seedlot No.	Locality	Latitude		Longitude		Altitude (m)
			deg.	min.	deg.	min.	
Casuarina cunninghamiana (= CC1)	13508	Augathella, Queensland	25	47	146	36	370
C. cunninghamiana (= CC2)	13519	Rollingstone, Queensland	19	1	146	20	20
C. cunninghamiana (= CC3)	15005	Nowra, New South Wales	34	52	150	27	70
C. glauca (= CG)	13987	Coffs Harbor, New South Wales	30	18	153	8	1
C. obesa (= CO)	14100	Wiluna, Western Australia	26	34	120	3	550
C. cristata (= CCR)	14843	Gilgandra, New South Wales	31	43	148	40	290
C. equisetifolia ssp. *equisetifolia* (= CE)	14196	Wangetti Beach, Queensland	16	41	145	35	2
C. equisetifolia ssp. *incana* (= CEI)	13990	North Stradbroke Island, Queensland	27	24	153	26	0
Allocasuarina torulosa (= AT1)	13337	Mount Lewis, Queensland	16	36	145	16	1000
A. torulosa (= AT2)	13992	North Stradbroke Island, Queensland	27	30	153	29	0

provenance, were combined in a 20-ml bottle, incubated in 10% C_2H_2 in air at 25°C, then assayed for acetylene reduction using gas chromatography according to Turner and Gibson (1980). Two replicates of 10 randomly selected seedlings without nodules were assayed as controls.

For each of the 3 transect replicates there were 240 different combinations of topographic position (6), soil depth (4), and host species or provenance (10). Rocks in the soil obstructed sampling so that only 586 out of a possible 720 replicated treatment combinations could be obtained. Compensation was made for missing replicates of treatment combinations by using the Generalized Least Squares method with type III sums of squares in the analysis of variance (SAS Institute 1982). Analysis of variance was used to determine variation associated with topographic position, soil depth and host plant species or provenance. Interactions between these three variables were also included in the analysis of variance. The three way interaction term was removed because it did not contribute significantly to total variation. Variation associated with transect replicates was considered in order to eliminate variation attributable solely to the random positioning of the transects.

Duncan's Multiple Range test was employed to identify statistically similar groupings of nodulation variables within topographic positions, soil depths and plant types. Linear regression analysis was used to examine the possibility of correlation between root length and nodulation variables (percentage of seedlings nodulated, no. of nodules, no. of seedlings nodulated and no. of nodules per nodulated seedling in a pot) and between shoot length and nodulation variables.

An additional experiment was conducted to determine whether crushed nodules from local *C. cunninghamiana* produced nodulation patterns in the 10 Casuarina and Allocasuarina sources similar to patterns produced by local soils in the baiting study. Seeds were sown densely in 2-liter cylindrical pots, 18 cm in diameter yielding 300 to 1500 seedlings per pot, depending on viability of a seed lot. Treatments were replicated twice. The seeds were sown in a mixture of equal volumes of Perlite and Vermiculite fertilized initially with 100 ml of quarter-strength Hoagland's solution (Hoagland and Arnon, 1950) and then, after plant shoots were 1 cm in height, biweekly with 100 ml of N-free, quarter-strength Hoagland's solution. Plants were inoculated when they reached 1 cm in height with 100 ml of a 1% saline solution containing fresh nodules homogenized for 1 minute in a blender to yield nodule pieces about 1 mm in average diameter. Each 100-ml suspension contained the equivalent of 2 g nodule dry weight. Seedlings were grown under the conditions described above and harvested 80 days after inoculation to determine nodulation.

Results

Highly significant ($p > 0.01$) differences in nodulation can be credited to topographic position, soil depth, host species or provenance, interaction of topographic position and soil depth, and interaction of topographic position and host species (Table 2). Significant differences in seedling survival could be attributed only to topographic position and seed source. Means for the number of surviving seedlings per pot were similar (28 to 30) for all topographic positions except the crest of the slope (38), which was significantly different (Table 3). Even with greater seedling survival rates in the soils of slope crests, the mean number of nodulated seedlings per pot was significantly smaller than at other topographic positions, negating any positive difference the higher mean seedling survival rate may have had on the analysis. Significant differences in seedling survival rate associated with species or provenance of Casuarinaceae were due to differences in seed viability of the CSIRO seed lots and were not apparently associated with any differences in nodulation (Table 5). More than 18,000 treatment and control seedlings survived to comprise the baiting study.

Seedling root length and shoot length were not significantly correlated with any nodulation variable and were discounted as a major factor influencing nodulation. Only two *C. cunninghamiana* seedlings became nodulated out of a total of 751 control seedlings in 30 pots of sterilized soil representing the entire range of soils sampled. This eliminates the possibility that *Frankia* contamination from the experimental procedure or the greenhouse environment was an important influence on seedling nodulation.

Table 2. Analysis of variance for percentage of seedlings nodulated per pot, number of nodules per pot, number of nodulated seedlings per pot, number of nodules per nodulated seedling in a pot, and total number of seedlings per pot

Source of variation	Degrees of freedom	Mean squares for				
		% seedlings nodulated	No. of nodules	No. of seedlings nodulated	No. of nodules per nodulated seedling	No. of seedlings
Topographic position	5	9,990**	3,861**	385.26**	37.753**	811*
Soil depth	3	3,485**	1,134**	83.40**	7.650**	442
Plant species and provenance	9	8,602**	5,773**	305.77**	22.268**	21,870**
Topo. × depth	15	1,061**	1,005**	56.22**	7.441**	374
Topo. × plant	45	1,157**	402**	26.63**	2.255**	408
Depth × plant	27	516	139	19.20**	1.567	249
Transect	2	585	1,163**	53.98**	8.419**	703
Error	480	353	152	10.09	1.094	329

Note: * = significant at the 5% level, ** = significant at the 1% level.

The mean number of seedlings nodulated per pot and the mean number of nodules per nodulated seedling for all species of Casuarinaceae decreased significantly with distance from the river bank (Table 3). This indicates a positive relationship between soil moisture and nodulation capacity of a soil at this locale. This idea is further supported by the significantly larger number of nodules per pot and per nodulated seedling that developed in soil samples from greater depths (Table 4), which we observed to have more moisture.

Interactions between topographic position and depth are illustrated in Fig. 1, which shows the largest number of nodules and nodulated Casuarinaceae seedlings occurred in soils from depths greater than 20 cm and in the alluvial sands. Soil samples taken adjacent to the river bank had similar infective capacity at all depths, more so

Table 3. Variation in nodulation of Casuarina and Allocasuarina seedlings associated with soils from different topographic positions

Topographic position	Number of pots	Mean no. of seedlings nodulated per pot	Mean no. of nodules per nodulated seedling	Mean no. seedlings per pot
Alluvial sand adjacent to river	87	5.7a*	2.1a	30a
Middle of alluvial sand	118	3.7b	1.2b	29a
Alluvial sand at base of slope	113	4.7c	1.3b	29a
Bottom of slope	103	1.3d	0.7c	28a
Midslope	87	1.3d	0.4d	29a
Crest of slope	79	0.7d	0.2d	38b

* Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 4. Variation in nodulation of Casuarina and Allocasuarina seedlings with depth of soils at Casuarina Sands

Soil depth (cm)	Number of pots	Mean No. of seedlings nodulated per pot	Mean no. of nodules per nodulated seedling	Mean no. of seedlings per pot
0–20	157	1.9a*	0.8a	30a
20–40	155	2.7b	0.9a	31a
40–60	146	3.4bc	1.0a	28a
60–80	129	4.1c	1.4b	31a

* Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$).

than other soils. At midslope and slope crest, the greatest number of nodules and nodulated seedlings per pot occurred in soil samples from 40 to 60 cm in depth, just above a heavy clay interpreted as an older, truncated buried soil (Van Dijk, 1959, 1969). Such paleosols often act as barriers to percolation of soil water, creating moister conditions in soils immediately above them.

In all soils, seedlings of three provenances of *C. cunninghamiana* together with seedlings of *C. glauca* Sieb. ex Spreng., *C. cristata* F. Muell ex Miq. and *C. obesa* Miq. developed in most cases significantly more nodules per pot and, in every case, developed significantly more nodules per nodulated seedling than did seedlings of two *C. equisetifolia* Forst. provenances (Table 5). Seedlings of two provenances of *A. torulosa* had significantly fewer nodulated seedlings per pot, nodules per nodulated seedling and lower nodulation percentages (< 1%) than all *Casuarina* host species, except one poorly-nodulated provenance of *C. equisetifolia*.

The interaction between host plant species or provenance and topographic position was statistically significant (Fig. 2). The *A. torulosa* provenances nodulated only in soils of the alluvial floodplain, primarily in soils sampled nearest the river, but only sparsely. Both *C. equisetifolia* provenances had greater nodulation in alluvial soils, exhibiting little nodulation in upland soil samples.

Nodulated seedlings of all *Casuarina* species reduced acetylene to ethylene (Table 6), though there was much variability in acetylene-reduction rates overall.

In the crushed-nodule experiment, inoculum from nodules of *C. cunninghamiana* growing at the

Table 5. Nodulation of Casuarina and Allocasuarina seedlings of different species and provenances in soils from Casuarina Sands

Plant species and source	Number of pots	Mean percentage seedlings nodulated	Mean no. of seedlings nodulated per pot	Mean no. of nodules per nodulated seedling	Mean no. of seedlings per pot
Casuarina cunninghamiana (CC2)	60	9a*	6.6a	1.6a	72a
C. cunninghamiana (CC1)	60	34b	5.4ab	1.6a	16de
C. cunninghamiana (CC3)	58	8ac	4.9b	1.4a	58b
C. glauca	60	11a	4.6bc	1.4a	42b
C. cristata	61	20c	3.4c	1.5a	17d
C. obesa	52	23b	2.1d	1.5a	9e
C. equisetifolia (CE1)	59	12a	1.5d	0.7b	13de
C. equisetifolia	59	4a	1.2de	0.4bc	32c
Allocasuarina torulosa (AT2)	58	< 1d	0.1e	0.1c	27c
A. torulosa (AT1)	60	< 1d	0.1e	0.02c	25c

* Means in a column followed by the same letter are not significantly different (α 0.05).

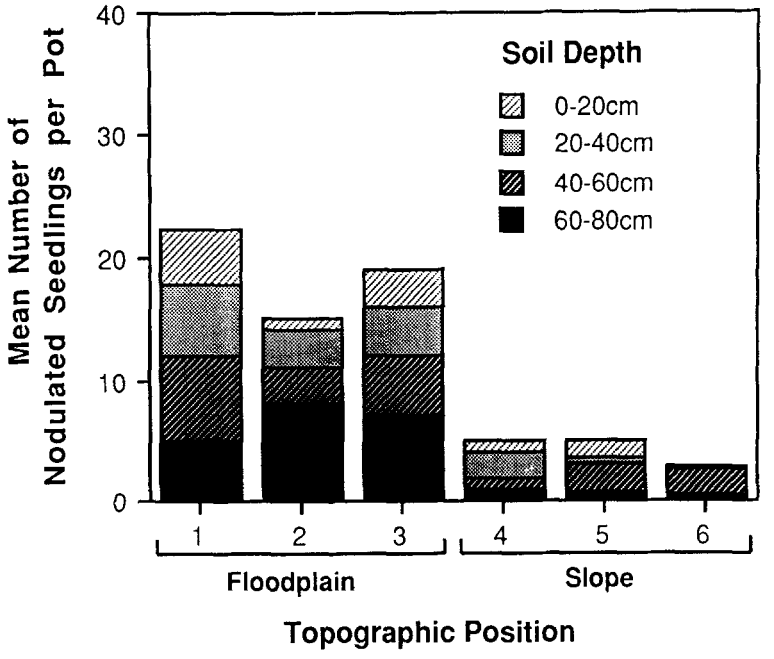

Fig. 1. Mean number of nodulated seedlings of Casuarinaceae per pot, for all species combined, by topographic position and soil depth. Topographic positions proceed from 1, the sample points adjacent to the river, to 6, the sample points at the crest of the adjacent slope.

study site produced results similar to those of associated soils. Percentages of seedlings nodulated per sample and the mean number of nodules per nodulated seedling were significantly less for *A. torulosa* provenances than for any of the *Casuarina* species (Table 7). However, use of crushed-nodule inoculum resulted in a larger percentage of seedlings being nodulated and more nodules per nodu-

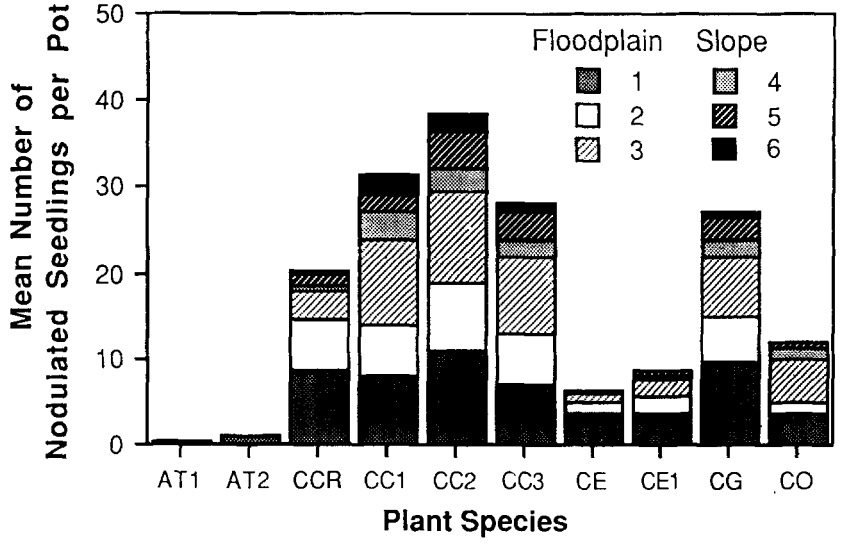

Fig. 2. Mean number of nodulated Casuarinaceae seedlings per pot, for all soil depths combined, by species and topographic position. Species designations are defined in Table 1. Topographic positions proceed from 1, the sample points adjacent to the river, to 6, the sample points at the crest of the adjacent slope.

Table 6. Acetylene reduction rates of nodulated Casuarina seedlings randomly selected from soil treatments. There are 10 pooled seedlings per assay

Tree species	Replicate	Mean no. of nodules per seedling	Nmoles C_2H_4 h^{-1} $plant^{-1}$
Casuarina cunninghamiana (CC1)	1	4.3	37
	2	3.6	12
C. cunninghamiana (CC2)	1	1.5	4
	2	4.2	10
C. cunninghamiana (CC3)	1	2.3	39
	2	3.1	5
C. glauca	1	5.0	5
	2	3.7	13
C. obesa	1	2.9	67
	2	2.1	6
C. cristata	1	1.0	3
	2	1.0	< 1
C. equisetifolia (CE1)	1	1.1	10
	2	1.0	5
C. equisetifolia	1	1.1	3
	2	1.1	2
Controls, plants with no nodules	1	0	< 1
	2	0	< 1

lated seedling than in the soil-baiting experiment. In addition there were significant differences in nodulation variables between *C. equiseifolia* provenances and other *Casuarina* species in the soil-baiting experiment but not in the experiment employing crushed-nodule inoculum.

Discussion

The results of this study indicate that there can be large differences in the infective capacity of soil with depth increments as small as 20 cm (Table 4) and with different topographic positions (Table 3). This includes soils sampled at the edge of the alluvial plain and at the bottom of the adjacent slope, positions which were separated by only a few meters distance. Therefore, assays of soil infective capacity should be conducted with consideration given to the possibility that topographic position, as well as edaphic factors such as soil depth and texture, can greatly influence nodulation capability of a soil sample.

Deeper soil strata in the alluvial sands had the greatest infective capacity of any soils sampled at this locale (Fig. 1). We observed that these strata were moister and cooler than surficial sands, and the relatively large amount of macropore space in sandy-textured soil affords the advantage of greater oxygen diffusion to deeper strata than in soils of finer texture. Thus, soils with the greatest nodulation capacity were those with the greatest capacity to afford adequate water, moderate temperature and aeration to roots and soil microorganisms. In a similar study, *Alnus glutinosa* (L.) Gaertn. seedlings germinated in minespoil from Illinois, USA produced less than 3% nodulated seedlings in spoil

Table 7. Nodulation of Casuarina and Allocasuarina seedlings inoculated with crushed nodule suspension from *C. cunninghamiana*. Mean values are for 50 seedlings samples from each of 2 pots

Plant species and source	Mean percentage of seedlings nodulated per sample	Mean no. of nodules per nodulated seedling
Casuarina glauca	100a*	4.0a
C. cunninghamiana (CC1)	90a	3.7a
C. cunninghamiana (CC3)	80a	2.1a
C. cunninghamiana (CC2)	60ab	2.0a
C. obesa	30b	4.0a
C. cristata	36b	3.3a
C. equisetifolia (CE1)	70ab	5.1a
C. equisetifolia	60ab	1.5b
Allocasuarina torulosa (AT1)	3c	1.0c
A. torulosa (AT2)	0	–

* Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$).

material from steep upper slopes, while 26% of the seedlings were nodulated in spoil material from a level terrace towards the bottom of the spoil heap (Dawson *et al.*, 1983). The level terrace afforded conditions suitable for infiltration and retention of water, favoring microbial and plant growth.

It has been established that the proximity in time and space of an actinorhizal host plant in soil can increase the infective capacity of that soil (Wollum *et al.*, 1968; van Dijk, 1979; Dawson and Klemp, 1987), even though presence of a host is not a necessary condition for soil infective capacity (Benecke, 1969; Bermudez de Castro *et al.*, 1976; Dawson *et al.*, 1983). It is possible that the greater infective capacity of alluvial floodplain soils in this study is owing, at least in part, to the presence of *C. cunninghamiana* and the continual input of *Frankia* propagules from sloughed nodules. *C. cunninghamiana* did not occur on the uplands at the Casuarina Sands study site.

Soils from this locale readily promoted nodulation of the Australian *Casuarina* species tested, although *C. equisetifolia* provenances developed significantly fewer nodules per nodulated seedling in these soils than the other species of *Casuarina* (Table 5). In contrast to the soil-baiting study, the *C. equisetifolia* ssp. *incana* provenance from North Stradbroke Island in Queensland, when inoculated with crushed nodules, produced many nodules similarly to *C. cunninghamiana* (Table 7). The latitudinal ranges of *C. cunninghamiana* and *C. equisetifolia* roughly correspond, although *C. cunninghamiana* occurs inland along freshwater streams, while the more salt-tolerant *C. equisetifolia* is a coastal species. *C. equisetifolia* ssp. *incana* occurs only along the coast of Queensland and northern New South Wales. The seed source of *C. equisetifolia* ssp. *incana* in this study is closer to the study locale, both geographically and latitudinally, than the *C. equisetifolia* ssp. *equisetifolia* provenance. Perhaps there is geographic variation in the capacity of *Frankia* to infect *C. equisetifolia*. It is possible that *C. equisetifolia* ssp. *incana* is more receptive to the local *Frankia* population of this study than ssp. *equisetifolia*. Fleming and coworkers (1988) found that *C. cunninghamiana* grew best when crushed-nodule inoculum source and seed source were from similar geographical origins. It is also possible that a selection or physiological change of *Frankia* occurred in the process of nodulation by *Frankia* resulting in a greater capacity of crushed-nodule inoculum to nodulate *C. equisetifolia* ssp. *incana* compared with soil from the same site.

Casuarina species and provenances used as hosts for *Frankia* in this study were from areas equally or more geographically remote from this locale than the *A. torulosa* provenances, yet they were more readily nodulated by baiting in local soils. We found that nodulation of *A. torulosa* was significantly less than nodulation of species of *Casuarina*. This is consistent with the findings of Reddell and Bowen (1986) that host-endophyte specificity was expressed mainly at the generic level in a crushed-nodule inoculation study involving 15 species in 3 genera of Casuarinaceae. The similarity of results obtained with crushed-nodule inoculum and soils from the same locale in our study indicate that local *C. cunninghamiana* may influence soil populations of *Frankia*.

Less than one percent of seedlings of the two provenances of *A. torulosa* became nodulated and only 6 of 117 soil samples yielded nodulated *A. torulosa* seedlings. It is notable that 4 of the 6 samples that promoted nodulation of *A. torulosa* were from soil depths between 20 and 60 cm at sample points adjacent to the river. The other 2 soil samples that yielded nodulated seedlings of baited *A. torulosa* were from the alluvial sands in the floodplain away from the river bank and from the 60 to 80 cm stratum. These 6 soil samples were the same samples that promoted the greatest nodulation of the *Casuarina* spp. Apparently these soils had the largest and most diverse *Frankia* populations, including strains capable of infecting *A. torulosa*, an upland species not native to the region.

A. littoralis (Salisb.) L. Johnson, *A. luehmanii* (R. T. Bak.) L. Johnson, and *A. verticillata* (Lam.) L. Johnson are native to dry upland areas in the A.C.T. In light of the apparent generic boundaries to cross inoculation within Australian Casuarinaceae (Reddell and Bowen 1986), one might expect upland soils to be more supportive of *Frankia* capable of infecting upland *A. torulosa*. However, the native *Allocasuarina* spp. in the A.C.T. are restricted in occurence and are neither widespread nor abundant, perhaps precluding abundant populations of associated *Frankia*. It is also possible that species of *Allocasuarina* are generally less easily nodulated than *Casuarina* species.

Seedlings of all *Casuarina* species nodulated in soil from this locale have the capacity to fix atmospheric nitrogen as indicated by acetylene-reduction capability (Table 6). However, for *C. cristata*, the presence of yellow cladodes in both the baited seedlings and those inoculated with crushed-nodule inoculum, together with their low rates of acetylene reduction, suggest that their symbiotic relationship with local *Frankia* was not highly effective. Overall, the rates of acetylene reduction obtained from assays of groups of 10 seedlings were highly variable for nodulated *Casuarina* seedlings. It was impossible to derive an assay sample for *A. torulosa* because of the low number of nodulated seedlings.

We have shown that soil depth and topographic position are factors that influence nodulation of Casuarinaceae in soils from a locale supporting *C. cunninghamiana*. This may explain, in part, the inconsistencies often encountered in field surveys for nodulation of Casuarinaceae in Australia and illustrate the probable localized promotional effects of soil moisture, temperature and aeration in an arid climate on infective *Frankia* in soil. We have also shown that either soil or crushed nodules obtained from a site supporting *C. cunninghamiana* promote nodulation of *Casuarina* species more readily than *A. torulosa* and that there may be a further degree of specificity of the local *Frankia* population within the genus Casuarina.

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