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Casuarina cunninghamiana Cladode Extracts Increase the *Frankia* Infectious Capacity of a Tropical Soil

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Abstract

Casuarina-infective *Frankia* (a symbiotic, root-nodule-forming diazotroph) and other *Frankia* can be localized near host plants, although the factors leading to this localization are not known. In this factorial experiment, *C. cunninghamiana* bait seedlings were inoculated with aqueous dilutions of soil harboring *Casuarina*-infective *Frankia* together with dilutions of homogenized *C. cunninghamiana* cladodes (photosynthetic branches). Seedlings were grown in one of two substrates: steam-pasteurized sand and gravel or unpasteurized Jamaican soil with little infectious capacity but of the same type and from the same general locale as the host-associated infective-soil inoculum. The addition of *Casuarina cunninghamiana* cladodes significantly increased nodule formation by soil-borne *Frankia* on the seedlings by a factor of 3.2. Similar additions of foliar homogenates of a sympatric check tree, *Terminalia catappa*, only slightly increased the nodulation capacity of the same soil inoculum (1.3x). In a separate experiment, a charcoal/clay mixture,

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added to bind soil and rhizosphere organic substances, significantly inhibited nodulation of *C. cunninghamiana* by a *Casuarina*-infective *Frankia* isolate. Results provide evidence that *Casuarina* hosts produce compounds that can significantly increase the infectivity of their microsymbiont *Frankia* in soil ecosystems.

Keywords: Actinorhiza, *Casuarina cunninghamiana, Frankia,* root nodules, symbiosis

1. Introduction

Frankia, a genus of nitrogen-fixing actinomycete, forms root nodules in symbiosis with 24 genera of actinorhizal plants (Baker and Schwintzer, 1990). Actinorhizal plants are important in the nitrogen economy of many diverse terrestrial ecosystems (Dawson, 1992; Chapin et al., 1994; Dommergues, 1997; Shumway, 2000). Host plant presence is the major factor in maintaining and amplifying *Frankia* populations in soil (Benson and Silvester, 1993). *Casuarina* spp. have restricted microsymbiont ranges (Torrey and Racette, 1989; Diem et al., 1983; Simonet et al., 1999). Even within their native habitats, nodulation of *Casuarina* and *Allocasuarina* species can be scarce or absent (Lawrie, 1982; Dawson et al., 1989). When planted outside their native range, *Casuarina* species usually do not become nodulated unless inoculated (Diem and Dommergues, 1990).

Casuarinas are exotic trees in Jamaica and are planted for soil stabilization, windbreaks, and ornamental purposes. *C. cunninghamiana*-infective *Frankia* were not detected at a number of Jamaican sites, (Zimpfer et al., 1997). However, Zimpfer et al. (1999) determined that *Casuarina*-infective *Frankia* were concentrated in a zone within a 20-m radius of *C. cunninghamiana* host trees in Jamaica. In this same study Zimpfer et al. (1999) obtained preliminary evidence to suggest that a *Casuarina*-infective *Frankia* strain was rendered more infective in an artificial soil when homogenates of green *C. cunninghamiana* cladodes were added. This led us to initiate studies under an expanded set of experimental conditions, including more-natural soil conditions, to further test the possibility that a *Casuarina* host species can produce substances that increase nodulation by its specific *Frankia* microsymbionts.

We hypothesized that compounds present in *Casuarina* tissue are capable of increasing nodulation of the host. We conducted an experiment to determine whether the addition of homogenized cladodes of *C. cunninghamiana* to seedlings inoculated with Jamaican soil containing *Casuarina*-infective *Frankia* would increase nodule formation. To determine if stimulation of

nodulation by dominant trees at this locale was restricted to compounds produced by *Casuarina* tissue alone, we tested whether foliar extracts of a common, sympatric tree would also increase soil infectivity. In another experiment we added charcoal and clay, capable of binding organic compounds, to infective soil supporting host seedlings to test for changes in soil nodulation capacity resulting from the adsorption of organic substances released into the host rhizosphere.

2. Materials and Methods

Plant culture

C. cunninghamiana plants were grown from seeds in 4 × 14-cm cone-shaped containers filled with one of two seedling growth substrates: 1) a steam-pasteurized 1:1:1 (v/v) mixture of vermiculite, sand, and gravel, 2) an unpasteurized soil with low background *Frankia* infectivity.

The soil was collected 50 m from mature *C. cunninghamiana* trees growing near Robin's Bay, Jamaica, and amended with steam-pasteurized vermiculite, sand, and gravel in a 1:1 (v/v) mixture to facilitate root washing. The Jamaican soil is a seawall stony clay, described as thin brown or reddish soil on hard coral limestone with poor water retention (Vernon, 1960) and with 4% organic matter (Zimpfer et al., 1999). In a previous baiting study at the same location, no *Casuarina*-infective *Frankia* were detected in this soil except immediately adjacent to *Casuarina* hosts (Zimpfer et al., 1999).

The "non-infective" soil included native biota but little or no infective *Frankia* in the experimental substrate. Thus treatment effects were tested in a biotically complex soil system. We chose to emulate soil ecosystems in this study using both soil and semisterile plant growth substrates open to colonization by air-borne microbes. Consistent with this approach we also employed an infective soil inoculum rather than a *Frankia* isolate in one of the experiments. This approach more-closely represents the normal infection milieu for the actinorhizal symbiosis. We have observed repeatedly and with many *Frankia* isolates that axenic techniques are incapable of promoting nodulation of *Casuarina*. However, once the axenic systems are compromised by exposure to airborne microbial contaminants, nodulation occurs. This indicates the probable importance of other microbes in the actinorhizal infection process, such as the helper bacteria in the *Alnus-Frankia* symbiosis (Knowlton et al., 1980; Knowlton and Dawson, 1983). The only successful report of axenic nodulation of casuarina by *Frankia* isolates employed powdered, activated charcoal in the agar rooting medium (Girgis, 1993). This suggests that host

plant roots may release not only nodulation-enhancing, but also inhibitory compounds. Perhaps elements of the soil ecosystem nullify the same inhibitory compounds apparently negated by charcoal adsorption.

Five soil samples were collected at the Jamaican coastal site to a depth of 10 cm using a sterilized, 2-cm diameter soil corer in each of 8, m^2 plots centered on points 50 m from the central *C. cunninghamiana* tree of a previous baiting study (Zimpfer et al., 1999). The plots were selected using a random set of azimuths. Cores were sealed in sterile plastic Whirl-Pak® bags (Nasco Corp., Fort Atkinson, WI) in the field. The bags were subsequently opened in a sealed room, placed immediately within a paper bag which was then sealed with staples to allow drying while minimizing aerial contamination. After drying for one week, the plastic bags containing soil were resealed, removed from the paper bags, and stored at room temperature for six months. Bioassays for nodulation with the same soil type from this locale had revealed that after two days of drying, infectious capacity of stored soils remained stable for 3 years. After six months the Jamaican soil was bulked and sieved through a 3-mm mesh screen to remove rocks and coarse organic material before mixing with vermiculite, sand and gravel.

Seeds of *C. cunninghamiana* were purchased from a commercial seed supplier who collected them from native stands in New South Wales, Australia. Seeds were surface-sterilized by submersion in a nylon-mesh bag weighted with glass beads and stirring in 30% v /v H_2O_2 in water for 20 min. This was followed by five similar 10-min rinses in distilled, deionized water (DDW), then seeds were immediately planted. Forty seeds, (±10) were planted approximately 2 mm deep in each container. Seedlings were grown at 23±5°C in a greenhouse during winter at the University of Illinois in Urbana. The natural photoperiod was extended to 16 h with 1000-watt high-pressure sodium lights providing 200–250 μmol m^{-2} sec^{-1} photosynthetically active radiation (400–700 nanometers wavelength or PAR) at the apex of seedling stems. The natural light during the diurnal cycle was also supplemented with artificial light automatically when sensors indicated that ambient light intensity had fallen below 400 mol m^{-2} sec^{-1}. The plants were watered as needed including with 1/8-strength (0.179 M NH_4NO_3) nutrient solution (Huss-Danell, 1978) added in lieu of water once each week. After 10 weeks, nitrogen was eliminated from the weekly application of nutrient solution allowing leaching of nitrate nitrogen from the pots in order to facilitate subsequent nodulation. Actinorhizal plant nodulation is inhibited by the availability of substrate N (Kohls and Baker, 1989).

Inoculation

At 12 weeks after germination *C. cunninghamiana* seedlings were inoculated with four dilutions of homogenized *C. cunninghamiana* cladodes and four dilutions of infective soil in the two soil substrates resulting in a 4 × 4 × 2 factorial experiment with containers completely randomized on greenhouse benches. At the time of inoculation, the roots of seedlings (5–20 per container) were extensive and had developed uniformly in mass and extent, despite the differences in numbers of seedlings, owing to the root-growth limitation imposed by the small container.

The infective soil was collected five m from the bole of a *C. cunninghamiana* tree, which soil had previously been shown to harbor *Casuarina*-infective *Frankia* (Zimpfer et al., 1999). The infective soil was collected at the same time as the poorly-infective soil and in the same manner, except that the distance from the trees was 5 m not 50 m. All soils were dried and stored under conditions described above. Cladodes were chosen as the host tissue type because they are deposited as litter under trees in large quantities and stimulated growth of *Frankia* isolates more than other tissues in preliminary studies (unpublished data available from authors). At the time of inoculation, bulked infective soil was homogenized by kneading in large plastic bags, after which 50 g were removed and placed in sterile beakers containing 300 ml of DDW and mechanically stirred for 30 minutes. After one minute, 100 ml were decanted, filtered through triple layers of cheesecloth, and added to 100 ml of DDW. From this original soil suspension a series of three ten-fold dilutions in DDW were prepared and seedlings in containers were inoculated with five ml of each soil suspension.

Cladode amendments

C. cunninghamiana cladodes were prepared by homogenizing 50 g of live cladodes in 1 L of DDW in a blender at high speed for three minutes, filtering the homogenate through a triple layer of cheese cloth, and freezing at –18°C prior to use. The cladodes were clipped from 1-m tall seedlings growing under the greenhouse conditions described above, but without nitrogen in the supplied nutrient solution. Cladode homogenates were chosen because of past success in promoting infection of actinorhizal hosts under a different, more-artificial experimental system employing a *Frankia* isolate, not native soil materials (Zimpfer at al., 1999). The cladode homogenates represent plant capacity to produce compounds that stimulate nodulation and do not simulate leaching from cladodes, roots, plant litter or exudation from roots in nature. However, the

results of the experiment with charcoal and clay soil amendments to nodulating *C. cunninghamiana* rhizospheres (this study) indicate the possibility of the release of compounds that stimulate nodulation from roots. We have also been able to stimulate the growth of *Frankia* isolates from *Casuarina* spp. with dilutions of root and senesced-cladode homogenates (unpublished data available from authors).

The seedlings were inoculated with the fresh weight equivalent of 0, 0.05, 0.5, or 5 g of *C. cunninghamiana* cladode tissue per container suspended in 10 ml of H_2O. All tissue homogenates were applied the same day as inoculation with infective soil, except for seedlings receiving 5 g, which were added at a rate of 0.5 g of tissue per container per day for ten consecutive days. There were four replicates of each combination of cladode homogenate concentration, infective soil dilution and seedling growth substrate for a total of 128 plants ($4 \times 4 \times 2 \times 4$ reps). Seedling containers were completely randomized on the greenhouse benches.

Twelve weeks after inoculation, the seedlings were harvested, their roots washed, and the nodules were counted. Each nodule formed was assumed to represent one infective unit of *Frankia*. In addition, the seedlings were placed in a 50°C drying oven for one week and the dry weight of plant biomass of each container was determined.

Statistical analysis

Analysis of variance (ANOVA) using a completely-randomized, factorial experimental design, was performed on the number of nodules per container to determine significant main effects and interactions of infective soil inoculum level, cladode homogenate level, and soil substrate. In addition, ANOVA was performed on seedling dry weight per container to determine the effects of the treatments on plant growth. Differences between individual means of treatments determined to contribute significantly to overall variation were tested for significance using least significant differences (LSD). We also correlated seedling dry weight per container with nodules formed per container. All statistical procedures and analyses were performed using SAS (SAS Institute, Inc. 1990).

Terminalia catappa foliar amendments

To determine whether the effects on nodulation of *C. cunninghamiana* photosynthetic tissue are different than the effects of plant photosynthetic tissue in general, we repeated the above experiment in a modified form with

leaf tissue of *Terminalia catappa,* an exotic tree occurring sympatrically with *C. cunninghamiana* at the study site in Jamaica. Again, *C. cunninghamiana* seedlings were grown in 4 × 14-cm cone-shaped containers filled with vermiculite, sand, and gravel, 1:1:1 (v/v). Seed preparation, growth, and culture were the same as described above. In a factorial experimental design, fourteen-week-old seedlings were inoculated with one of four levels of homogenized *T. catappa* leaves (0, 0.05, 0.5, 5 g of tissue per container) and one of three dilution levels of *Casuarina*-infective soil (0.4, 0.04, 0.004 g soil per container). *Terminalia catappa* leaves were harvested from a 3-m tall specimen growing in the University of Illinois greenhouses. Plant tissue amendments and inoculation with infective soil were as described above.

Twelve weeks after inoculation, the *C. cunninghamiana* bait seedlings were harvested, their roots washed, and the nodules were counted. Analysis of variance was performed on the number of nodules per container to determine significant main effects and interactions of soil inoculum level and tissue homogenate level. For significant main factors, differences between means of treatment levels were determined (LSD).

Effects of charcoal and clay soil amendments on nodulation

In order to determine if a decrease in organic compounds in the host rhizosphere would result in a decrease in nodulation, we conducted a factorial experiment with all four combinations of + or –charcoal and + or –clay, as well as three levels of *Frankia* added to the *C. cunninghamiana* bait-seedling growth substrate. Charcoal and clay have the ability to adsorb phenolics and organic compounds released from litter and vegetation (Siantar et al., 1994; Pietikainen et al., 2000).

Frankia was grown in the medium of Burggraaf and Shipton (1982) with propionic acid as the carbon source. Prior to use as inoculum, *Frankia* colonies were washed using centrifugation at 2,000 rpm (650 × G) in a clinical centrifuge for 15 min. three times before volume determination. Before inoculation, *Frankia* colonies were homogenized using a sterile glass tissue grinder.

As before, *C. cunninghamiana* seedlings were grown in 4 × 14-cm cone-shaped containers. Half of the containers were filled with a 1:1:1 mixture (v/v) of vermiculite, sand, and gravel and half of the containers were filled with a 3:3:3:1 mixture (v/v) of vermiculite, sand, gravel and granulated charcoal. Seedling preparation, growth and maintenance were the same as described above. Ten-week-old seedlings were inoculated with 0.5, 0.05 and 0.005 μl (packed cell volume) of *Frankia* isolate CjI82 001 (Diem et al., 1983) suspended in 5 ml DDW. Immediately following *Frankia* inoculation, 0.0625 g of

Montmorillonite clay suspended in 4 ml of DDW was added to half of each seedling treatment. Five replicate tubes were prepared for each treatment combination.

Ten weeks after inoculation, the seedlings were harvested, their roots washed, and the nodules were counted. Analysis of variance was performed on the number of nodules per container to determine significant treatment effects.

In addition, separate factorial analyses were performed at each level of *Frankia* inoculum for the four possible combinations of + or –charcoal, + or –clay. This additional set of analyses was performed to allow for the detection and elimination of data from a given inoculum level that exceeded saturation levels of root nodulation sites, thus precluding detection of treatment effects. For significant treatment effects, differences between individual means of a significant treatment variable were determined (LSD).

Table 1. Summary ANOVA table for number of nodules formed per container of *Casuarina cunninghamiana* seedlings grown in either steam-pasteurized sand and gravel or an unpasteurized, low-infectivity soil. Tubes were inoculated with four levels (0.4, 0.04, 0.004 and 0.0004 g per container) of infectious soil collected 5 m from mature nodulated *Casuarina* trees and four levels (0, 0.05, 0.5, 5 g per tube) of homogenized *Casuarina* cladode tissue.

Source	DF	Mean squares	F value	P>F
Infectious-soil dilutions	3	2553.28	64.25	0.0001
Cladode dilutions	3	576.59	14.51	0.0001
Infectious soil × Cladode dilutions	9	381.18	9.59	0.0001
Growth substrate	1	634.57	15.97	0.0001
Infectious-soil dilution × Growth substrate	3	8.76	0.22	0.8820
Cladode homogenate × Growth substrate	3	35.03	0.88	0.4536
Infectious soil × Cladode homogenate × Growth substrate	9	21.44	0.54	0.8423
Error	96	39.74		
Total	127	4250.58		

3. Results

Effects of C. cunninghamina cladode tissue on nodulation

Infective soil, levels of cladode tissue, and type of growth substrate, as well as an interaction between levels of infective soil and cladode tissue, significantly affected nodulation (Table 1). Nodules did not form in control containers filled with vermiculite, sand, and gravel, whereas in control containers of seedlings grown in the poorly-infective soil, a mean background level of 3.7 nodules per container were formed, which was a statistically significant but small difference relative to other treatment effects. Increasing levels of cladode homogenates and infective soil applied to seedlings grown in both growth substrates combined resulted in increased nodule formation (Fig. 1). A much greater increase in nodulation occurred in containers inoculated with the highest level of infective soil and cladodes in comparison with seedlings inoculated with the lowest levels of infective soil and the highest level of cladodes (Fig. 1). Analysis of variance performed on the dry weight of seedlings per container revealed that neither infective soil, cladode tissue, nor growth substrate affected the mean dry weight of seedlings per container significantly. This suggests that the mass of host plant tissue was not a factor in regulating the number of nodules formed per plant, and that nutrient differences in the cladode tissue, soil substrate and soil inoculum did not significantly affect host plant growth.

Effects of Terminalia catappa leaf tissue on nodulation

The number of nodules formed per container of seedlings grown in vermiculite, sand, and gravel inoculated with infectious soil and homogenized *T. catappa* leaf tissue revealed that the level of infective soil and leaf tissue affected nodulation significantly (Table 2). Increasing levels of homogenized leaves and infective soil applied to seedlings grown in vermiculite, sand, and gravel resulted in increased nodule formation (Fig. 2). Containers filled with vermiculite, sand, and gravel inoculated with 0.4 g of infective soil per container and inoculated with the fresh weight equivalent of 5 g of *Casuarina* cladode tissue had 2.12-fold more nodules than control seedlings without cladodes (Table 3). In contrast, seedlings grown in vermiculite, sand, and gravel and inoculated with 0.4 g of infective soil and inoculated with the fresh weight equivalent of 5 g of homogenized *T. catappa* leaves, formed 1.32-fold more nodules than control seedlings without added leaves (Table 3).

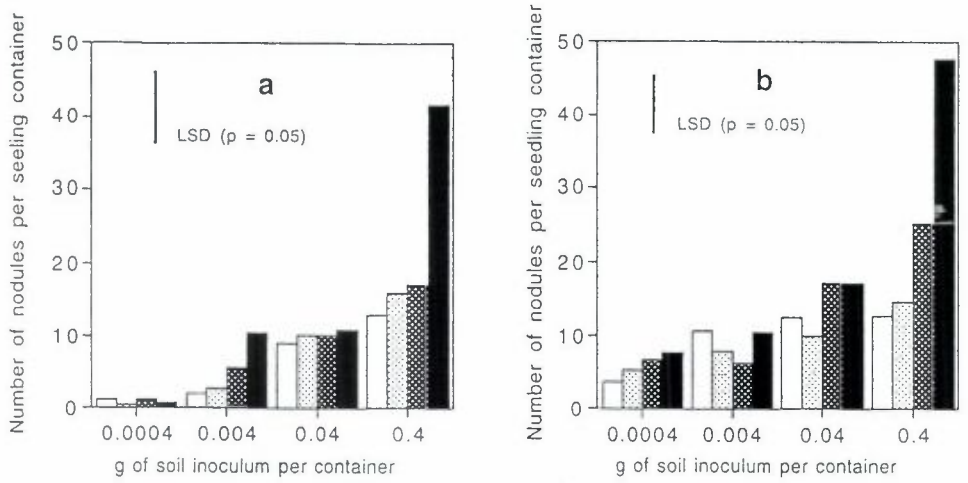

Figure 1. Effects of soil and cladode tissue on nodulation of *Casuarina cunninghamiana* bait seedlings growing in a) containers filled with steam pasteurized sand and gravel or b) low-infective soil added to steam pasteurized sand and gravel. Seedlings were inoculated with infective soil collected 5 m from mature *C. cunninghamiana* trees and with □ 0 g, ▒ 0.05 g, ■ 0.5 g or ■ 5 g of homogenized *C. cunninghamiana* tissue. Bar represents least significant difference at p = 0.05 (n = 4).

Table 2. Summary ANOVA table for number of nodules formed per container of *Casuarina cunninghamiana* seedlings grown in steam-pasteurized sand and gravel. Containers were inoculated with three levels (0.4, 0.04, and 0.004 and g per tube) of infectious soil collected 5 m from mature nodulated *Casuarina* trees and four levels (0, 0.05, 0.5, 5 g per tube) of homogenized *Terminalia catappa* leaf tissue.

Source	DF	Mean squares	F value	P > F
Infectious-soil dilution	2	24360.80	146.71	0.0001
Leaf homogenate dilution	3	539.57	3.25	0.0298
Infectious soil × Leaf homogenate dilution	6	254.48	1.53	0.1879
Error	48	166.05		
Total	59	25320.91		

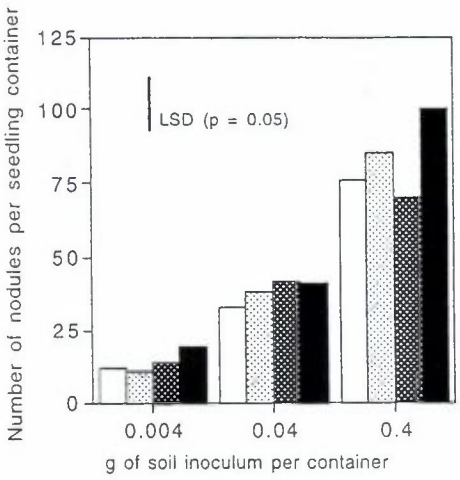

Fig. 2.

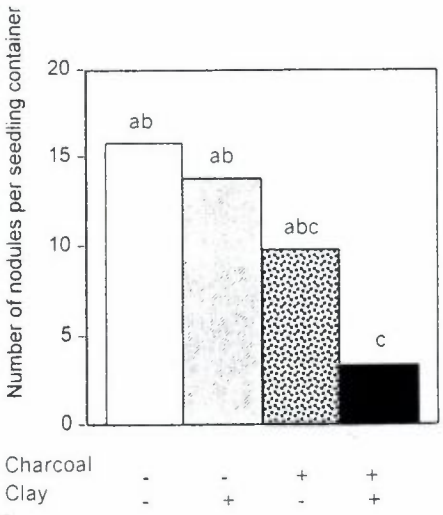

Fig. 3.

Figure 2. Effects of soil and cladode tissue on nodulation of *Casuarina cunninghamiana* bait seedlings growing in containers filled with steam pasteurized sand and gravel. Seedlings were inoculated with soil collected 5 m from mature *C. cunninghamiana* trees and with ☐ 0 g or ▨ 0.05 g or ■ 0.5 g or ■ 5 g of homogenized *Terminalia catappa* leaf tissue. Bar represents least significant difference at p = 0.05 (n = 5).

Figure 3. Effects of clay and charcoal on nodulation of *Casuarina cunninghamiana* bait seedlings growing in containers filled with steam pasteurized sand and gravel. Seedlings were inoculated with 0.005 µl (packed cell volume) of *Frankia* isolate CjI82 001. The columns marked with different letters are significantly different (LSD, p = 0.05; n = 5).

Effects of charcoal and clay on nodulation

The mean number of nodules formed per container of seedlings was significantly different for charcoal amendment only at the lowest level (0.005 µl) of *Frankia* inoculum (Table 4). An LSD means comparison (Fig. 3) revealed that only seedlings in the treatment with both added charcoal and clay formed significantly fewer nodules than the control seedlings.

4. Discussion

Compounds present in *C. cunninghamiana* cladode tissue extracts can apparently increase the nodulation capacity of soil containing naturally-

Table 3. Mean number of nodules formed per container of *Casuarina cunninghamiana* seedlings grown in steam-pasteurized sand and gravel. Containers were inoculated with either 0.4, 0.04, or 0.004g of infectious soil collected near *Casuarina* trees and four levels (0, 0.05, 0.5, 5 g per container) of homogenized *C. cunninghamiana* cladode tissue or *Terminalia catappa* leaf tissue.

g tissue added per container	Tissue source	
	Percent of nodules formed per container compared to control	
	C. cunninghamiana cladodes	*T. catappa* leaves
5	212 a*	132 a
0.5	137 b	102 b
0.05	104 b	110 ab
0 (control)	100 b	100 b

*Values within columns proceeded by different letters are significantly different at $p = 0.05$. Differences were determined on the actual means.

Table 4. Summary ANOVA table for number of nodules formed per charcoal and clay amended containers of *Casuarina cunninghamiana* seedlings. All seedling containers were inoculated with 0.005 µl (packed cell volume) of *Frankia* isolate CjI82 001. Seedling containers were filled with either steam-pasteurized vermiculite, sand and gravel or steam-pasteurized vermiculite, sand and gravel with 10% v/v charcoal and with half of each of these treatments receiving 0.0625 g of montmorillonite clay (n = 5).

Source	DF	Mean squares	F value
Charcoal	1	336	11.07**
Clay	1	88.2	2.91
Charcoal × clay	1	24.2	0.80
Error	16	30.35	
Total	19	478.75	

**Significant at p = 0 .01.

occurring *Frankia,* while the addition of organic-binding agents to the host rhizosphere decreases nodulation. The increase in nodulation capacity by the addition of extracts from homogenized *C. cunninghamiana* tissue may result

from the stimulation in growth of *Frankia*, the stimulation of "helper" bacteria, the inhibition of competitors of *Frankia*, the enhancement of signaling by molecules important in the nodulation process, the stimulation of root hair curling or fine root production, or perhaps some combination of these influences (Knowlton et al., 1983; Kapulnik et al., 1987; Benoit and Berry, 1997; Gauthier et al., 2000). Nickel et al. (1999, 2001) found that *Alnus*-infective *Frankia* are able to grow saprophytically in incubated soils and that the addition of *Alnus* leaves to incubated soils can increase both the growth and infectivity of *Alnus*-infective strains of *Frankia*.

Phenolics in plant tissue (Harborn, 1973) and soils (Whitehead, 1964; Li et al., 1970; Shindo et al., 1978; Whitehead et al., 1983) may influence the interaction between plants and soil microorganisms. In the *Rhizobium*-legume symbioses, flavonoids, anthocyanins, betains, and jasmonic acid affect nodulation genes (Hungria et al., 1992; Phillips et al., 1992; Rosas et al., 1998). Kapulnik et al. (1987) demonstrated a correlation between the concentration of specific host flavonoids and the degree of nodulation in the *R. meliloti*-alfalfa system.

Perradin et al. (1983) and Vogel and Dawson (1986) determined that phenolics significantly stimulate or inhibit the *in vitro* growth of *Frankia* strains as well as alter their morphological development. Commercial flavonoids and clover exudates increase the rate of growth of *Rhizobium leguminosarum* strains (Janczarek et al., 1996). Research by Selim et al. (1996) revealed that growth of *Frankia* associated with *Casuarina* could be promoted with various fatty acids when grown in pure culture.

Flavonoids have been found to influence the actinorhizal symbiosis (Benoit and Berry, 1997; Laplaze et al., 1999). Casuarina trees are infected intracellularly following root hair curling, which is induced by an unknown *Frankia* signal (Laplaze et al., 1999). Similarities in the infection process between some actinorhizal plants and legumes have led to the hypothesis that flavonoids act as actinorhizal plant signals, activating the production of *Frankia* root hair-deforming factor (Prin and Rougier, 1987; Van Ghelue et al., 1997). Thus, it is plausible that host tissue of actinorhizal plants and their relatives produce compounds that mediate host/symbiont signaling.

Seedlings grown in low-infectivity soil had an average of 3.7 more nodules per container than seedlings grown in vermiculite, sand and gravel, representing a low background level not detectable in a previous study (Zimpfer et at., 1999). This accounts for the significant difference for growth substrate in the analysis of variance (Table 1). However, this difference was uniform across treatment combinations and was small with respect to the magnitude of response to

cladode and soil dilution treatments (Figs. 1–3). So the treatment results for the two growth substrates are combined in interpretive figures and discussion.

A lack of inhibition of nodulation by the biotically complex, low-infectivity soil substrate suggests that competition from associated soil organisms does not alter infectivity of soils harboring *Frankia*. Furthermore, if competition had significantly affected the infectivity of soil, less nodulation in unpasteurized Jamaican soil than in pasteurized vermiculite, sand and gravel would have been expected.

The substantially greater increase in nodulation of seedlings inoculated with 0.4 g of infective soil and extracts from 5 g of homogenized cladode tissue (212% that of control seedlings; Table 3) compared to seedlings inoculated with 0.4 g of infective soils and extracts from 5 g of homogenized *T. catappa* leaves (132% that of control seedlings; Table 3) indicates that the increase in nodulation capacity from *C. cunninghamiana* cladodes is not the same for photosynthetic tissue from another angiospermous plant and indicates the possibility that the response is host-specific.

Added clay and charcoal reduced nodulation, indicating that compounds released from *Casuarina* tissue in this study increased nodulation. However, charcoal and clay inhibited nodulation only at the lowest dilution level of added *Frankia*. The number of units of infective *Frankia* was less at this inoculum level than at the other more-concentrated soil inoculum levels. This probably made it more responsive to treatments than the other more-concentrated inoculum levels that provided numbers of infective units exceeding the number of root infection sites. We do not know whether compounds in cladodes differ from those apparently released from roots in our experiments. Furthermore, in the study with added charcoal and clay, we used a *Frankia* isolate rather than soil as a *Frankia* source, which makes direct comparison of results of this study with those employing soil-born *Frankia* problematic.

Frankia's ability to proliferate in *Alnus glutinosa* rhizospheres (Vergnaud et al., 1985) and its localization near *C. cunninghamiana* (Zimpfer et al., 1999) suggest that it may be favored in host rhizospheres. Smolander et al. (1990) found *Frankia* nodulation capacity is increased in the rhizosphere of *Betula pendula* Roth., a member of the same family as *Alnus* spp. Similarly, Paschke and Dawson (1992) found that *Frankia* nodulation capacity is stimulated in soil beneath *Betula nigra*. Using *Gymnostoma poissonianum* as bait plants, Gauthier et al. (2000) determined that a soil from the rhizosphere of a non-nodulated, endemic rhamnaceous species, *Alphitonia neocaledonica*, harbored more infective *Frankia* than the rhizospheres of *Pinus caribea* or bare soil. Thus, nonactinorhizal plants in the same families as actinorhizal genera can also stimulate *Frankia* infectivity.

The results of our experiments support the hypothesis that casuarina host plants may release compounds that favor the infectivity of their specific microsymbionts. The similarity of results in both pasteurized artificial soil and the poorly-infective soil substrate suggests that compounds in host cladode tissue can stimulate the nodulation process when the soil biota originates from either a natural soil or from an isolate of *Frankia* in the presence of microbes from background sources in a greenhouse.

The infectivity of soils containing *Casuarina*-infective *Frankia* can be increased by the addition of homogenized *C. cunninghamiana* cladode tissue, while a decrease in host plant nodulation can be achieved by the addition of charcoal and clay to the growth substrate of seedlings inoculated with a *Casuarina*-infective isolate. We therefore propose that *Casuarina* hosts may be increasing their own nodulation numbers through the release of a chemical stimulus from host tissue.

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