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# Rhizosphere Microbiota and *Frankia-Casuarina* Symbiosis

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## ABSTRACT

Four *Frankia* strains were used to inoculate three *Casuarina* species in sterilized and unsterilized soils. Plants were analyzed for their growth performance as well as their enzymatic activities after three months of inoculation. Catalase, lipid peroxidation (malondialdehyde), free amino acids, and proline were measured for the treated plants after three months of inoculation. Only nodulation and plant performance were determined after three, six, and nine months of inoculation. The magnitude of plant performance was as follows: uninoculated plants in unsterilized soil ≥ inoculated plants in unsterilized soil > inoculated plants in sterilized soil. The indigenous *Frankia* populations may be more adapted to benefit from the intact soil biota found in the unsterilized soils than the introduced *Frankia* strains except in few treatments. The increase in catalase (CAT) and decrease in malondialdehyde (MDA) and proline content of plants in unsterilized soil indicate that indigenous *Frankia*, in combination with other accompanying microorganisms, are more beneficial for plant performance. Soil biotic community may produce the required factors for plant protection and successful growth conditions, in combination with the indigenous infective and effective *Frankia* strains.

**Keywords**: antioxidant enzymes, *Casuarina* (Casuarinaceae), *Frankia* (Frankiaceae), microbiota, nodulation, rhizosphere, symbiosis, unstreilized soil.

## INTRODUCTION

Rhizosphere microbes may improve the uptake of nutrients by plants and/or produce plant growth promoting compounds, they also protect plant root surfaces from colonization by pathogenic microbes through direct competitive effects and production of anti-microbial agents (Glick, 1995). These plant growth-promoting bacteria can enter into a symbiotic relationship with plants (i.e. *Rhizobium*-legume and *Frankia*-actinorhizal plant symbioses). There are also non- symbiotic, free-living, soil bacteria that can promote plant growth by other means (Glick, 1995).

*Frankia* can be found as a symbiont of actinorhizal plants, an associate of non- host plants or as a saprophyte. There is a regulated series of events leading to the association between infective *Frankia* and compatible host plant and the subsequent formation of root nodules on its roots (Maunuksela, 2001). There are other studies demonstrating a correlation between nodulation capacities and soil properties such as moisture, organic matter and pH (Righetti *et al.*, 1986; Dawson *et al.*, 1989; Smolander *et al.*, 1988; Young *et al.*, 1992).

Many plant metabolic processes produce active oxygen species (AOS), even in normal conditions. Biotic and/ or abiotic stresses can increase AOS concentrations that result in oxidative damage of cells (Mittler, 2002). Therefore, investigating the levels of antioxidant enzymes was one of the specific aims of the current study in addition to some other metabolic products such as proline, soluble proteins, and malondialdehyde as indicators of plant performance under the experimental conditions.

Moreover, the effect of soil biotic factors in unsterilized soil (i.e. soil microbiota), under different *Casuarina* spp, on the nodulation capacities of natural (indigenous) and cultured (exogenous) *Frankia* populations and their host plant performance was also targeted.

## MATERIALS AND METHODS

### Experimental setup

Four *Frankia* strains were used: UGL020601, UGL020602 (Sayed *et al.*, 1998), 0RS021001 (Diem *et al.*, 1983), and HFPCcI3 (Zhang *et al.*, 1984). *Frankia* strains were subcultured in liquid BAP medium (Murry *et al*, 1984) and left to grow at 28°C for 4 weeks. Protein content was adjusted to 5.5 µg $ml^{-1}$ for each strain (Hooker, 1987).

The above *Frankia* inocula were used to inoculate *C. equisetifolia, C. cunninghamiana* and *C. obesa* plants in sterilized and unsterilized soil with a control treatment (uninoculated, unsterilized soil). All *Casuarina* seeds were collected from the Division of Forestry, Australian Tree Seed Center (Canberra, Australia). Seeds were surface sterilized with $H_2O_2$ (30%) for 3 - 5 minutes and rinsed several times with sterile distilled water. Seeds were germinated in Vermiculite™ in trays sterilized by swabbing with methanol several times and then left to dry. Six-week old *Casuarina* seedlings with uniform size were then transplanted in 1 kg of sand/ clay soils (3: 1) in 12 x 25 cm polyethylene bags. Young plants were watered every third day with Crone's solution and "A-Z" micronutrient solution (Sayed *et al*., 2000). Plants were grown for 3, 6 and 9 months in a controlled "Fitoclima 801" growth cabinet (Snijders Scientific, the Netherlands). Temperature was maintained at 29 ± 2 °C, humidity at 50 ± 5 % and with a 16 hr light period (5K lux).

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Plant inoculation procedure was carried out according to Baker & Shwintzer (1990) as follows: 6 week old *Casuarina* seedlings were inoculated by injecting 10 ml from each *Frankia* inoculum (5.5µg protein $ml^{-1}$) into the surrounding soil (rhizosphere). The inoculation procedure was repeated again after one week.

Treatments were as follow: uninoculated plants in unsterilized soil (Control); inoculated plants in sterilized soil (T1); and inoculated plants in unsterilized soil (T2). Seedlings were examined carefully for nodulation after 4 weeks of inoculation and then weekly up to 12 weeks. Plants were harvested after 3, 6 and 9 months, washed carefully under tap water and dried on a paper towel. The number of nodules, shoot height and root length were recorded. Plants were then dried in an oven at 80 °C for 48 hours. Plant shoot, root, and nodule dry weights were determined. Finally, plant total nitrogen content was determined according to Nelson & Sommers (1973), and Sayed (1995) using the micro-Kjeldahl technique.

In addition, the following analyses were carried out for the treated plants after 3 months only.

**Enzyme extraction**

The needles of *Casuarina* were used for plant enzyme analysis. Sample preparation was described by Mukherjee & Choudhuri (1983). A leaf sample (0.5gm) was frozen in liquid nitrogen and finely ground using pestle and mortar. The frozen powder was added to 10 ml of 100 mM phosphate buffer ($KH_2PO_4$ / $K_2HPO_4$), pH 7.0, containing 0.1 mM $Na_2EDTA$ and 0.1 gm of polyvinylpyrrolidone. The homogenate was filtered through cheese cloth and centrifuged at 15,000 rpm at 4° C for 10 minutes. The supernatant was re- centrifuged at 18,000 rpm at 4 °C for 10 minutes. The resulted supernatant was collected and stored at 4 °C for enzyme assay.

**Determination of antioxidant enzyme activities**

Catalase (EC 1.11.1.6) activity was assayed in a 3 ml reaction solution composed of 50 mM phosphate buffer (pH 7.0), 30% (w/ v) $H_2O_2$ and 0.5ml of plant extract (Aebi, 1984). The activity of catalase was estimated as the decrease of absorbency at 240 nm due to $H_2O_2$ consumption compared to free enzyme extract sample (Havir & Mellate, 1987).

**Determination of lipid peroxidation**

The level of lipid peroxidation was measured as the amount of malondialdehyde (MDA) produced resulting from the peroxidation of unsaturated fatty acids (Heath & Packer, 1968). A fresh leaf sample (0.5gm) was homogenized in 10 ml of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000rpm at 4°C for 10 minutes. To 2.0ml aliquot of the supernatant, 4.0ml of 0.5% thiobarbituric acid in 20% TCA was added. The mixture was heated at 95°C for 30 minutes and then quickly cooled in an ice bath, then centrifuged at 10,000 rpm for 10 minutes. The absorbency of supernatant was recorded at 532 nm. The value for non-specific absorbency at 600 nm was subtracted. The MDA content was calculated using its extinction coefficient of 155 nmol $cm^{-1}$ and expressed as nmol (MDA) $gm^{-1}$ fresh matter (Ismail *et al*., 2004).

**Determination of water-soluble protein**

Powder of tissue samples (50 mg each) was boiled in 10ml distilled water for two hours. After cooling, the water extract was centrifuged, (12000 rpm for 15 minutes) the supernatant decanted, and the pellet completed to a definite volume using distilled water. Soluble proteins were then determined according to Lowery *et al* (1951).

Reagent **A:** 2gm of sodium carbonate in 100ml of 0.1 N NaOH (2 % $Na_2CO_3$ in 0.1 N NaOH).

Reagent **B:** 0.5 gm $CuSO_4.5H_2O$ in 1% sodium-potassium tartarate. The alkaline reagent solution consists of 50ml of reagent **A** and one ml of reagent **B**. This reagent should be always freshly prepared.

Five ml of the alkaline reagent solution were added to 0.1ml of the test solution (plant water extract) in a clean test tube, mixed thoroughly using vortex mixer and allowed to stand at room temperature for at least 10 minutes. Then 0.5 ml of the diluted "Folin-Ciocalteau" reagent (1:1 v/ v) was added to the above mixture, and mixed immediately. After 30 min, the extinction against appropriate blank was measured at 700nm. A calibration curve was plotted using egg albumin and the data were expressed as mg protein/ gm dry matter.

**Determination of proline**

Free proline was determined according to Bates *et al*. (1973). A definite weight of macerated dry matter tissue was homogenized in 5 ml of 3% sulfosalicylic acid, then filtered through Whatman® no. 2 filter paper. Two ml of the filtrate were mixed with 2ml glacial acetic acid and 2ml of acid ninhydrin in a test tube for one hour at 100°C. The reaction mixture was extracted with 4ml toluene, mixed vigorously in a test tube for 15 - 20 second. The chromophore containing toluene was aspirated from aqueous phase and warmed to room temperature. The absorbency was measured at 520nm using a standard curve and calculated on a dry weight basis as mg proline/ $gm^{-1}$ dry matter.

**Determination of total free amino acids**

Free amino acids were extracted from plant tissues and determined according to the method of Moore & Stein (1948). However, in this method traces of proline and hydroxy proline are encountered. A calibration curve was constructed using glycine. The concentration of the free amino acids was calculated as mg $gm^{-1}$ dry matter.

## RESULTS

### Nodulation

In general, after three months, the number of nodules (Fig. 1) and nodule dry weight (data not shown) were lower, for all *Casuarina* spp in inoculated sterilized and unsterilized soil, than the control plants (uninoculated plants in unsterilized soil). The magnitude was as follows: Control (uninoculated plants in unsterilized soil) ≥ inoculated plants in unsterilized soil > inoculated plants in sterilized soil. On the other hand, nodules as a percentage of whole plant dry weight increased significantly for *C. obesa* and *C. cunninghamiana* than control plants for all treatments. For *C. equisetifolia* the values varied significantly with variation in the used *Frankia* strain.

Nodulation capacity was approximately 2 times higher, for inoculated plants in unsterilized soil, than plants in sterilized soil and inoculated with the same *Frankia* strains after 3, 6 and 9 months (Fig. 1). Larger and healthier nodules were observed in all *Casuarina* plants in unsterilized soil than those in sterilized soil especially after 9 months (Fig. 2).

### Plant performance

Generally, higher dry weights were recorded for all *Casuarina* plants, inoculated with different *Frankia* strains in unsterilized soil, than sterilized soils, after 3, 6 and 9 months (Fig. 3).

Consequently, the lowest root/ shoot ratios were recorded for all *Casuarina* plants grown in unsterilized soil, inoculated with different *Frankia* strains, after 3, 6 and 9 months (data no shown). Higher shoot heights (and more vigorous growth) were also recorded for plants in unsterilized soil than those in sterilized soil inoculated with the same *Frankia* strains (Fig. 4).

Finally, total N for plants grown in unsterilized soil was higher than those grown in sterilized soil for 3, 6 and 9 months with all *Frankia* strains (Fig. 3).

### Biochemical analysis of treated Casuarina plants

The data of antioxidant enzymes, lipid peroxidation, total free amino acids, and proline contents of the treated *Casuarina* plants are presented in Fig. (5). It is worth mentioning that the absorbency of replicates, of enzyme assays, had no differences and gave the same values when executed (i.e. SD values= 0.0).

*(1) Antioxidant enzymes*

Fig. (5) shows remarkable increase in the activity of catalase enzyme for all *Casuarina* spp. planted in unsterilized soil than those in sterilized soil, with the exception of *C. equisetifolia* and *C. cunninghamiana* when inoculated with *Frankia* strains ORS021001 and HFPCcI3, respectively.

*(2) Lipid peroxidation*

The value of MDA (malondialdehyde) decreased greatly within all *Casuarina* spp. in unsterilized-than in sterilized soil (Fig. 5). The exceptions were *C.*

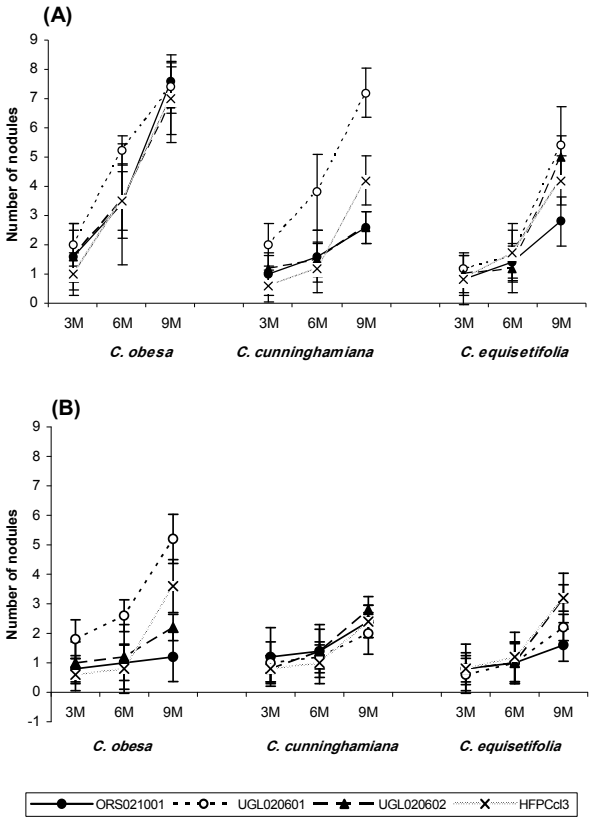


**Figure (1)**: Change in number of nodules for different *Casuarina* spp. inoculated with different *Frankia* strains, after 3, 6 and 9 month in unsterilized soil (A) and in sterilized soil (B).

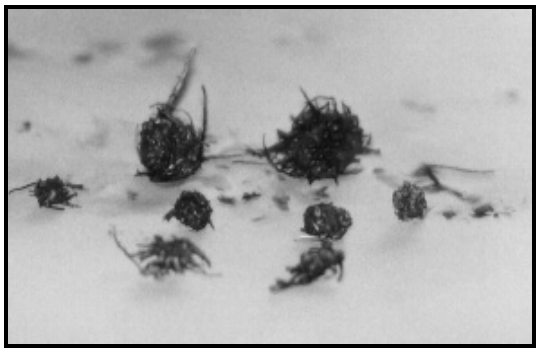

**Figure (2)**: Differences between nodules formed in unsterilized soil (large) and in sterilized soil (small) from 9 months old *Casuarina obesa*, inoculated with *Frankia* strain ORS021001.

*equisetifolia* and *C. cunninghamiana*, that were inoculated with *Frankia* strains ORS021001 and HFPCcI3, respectively.

*(3) Total free amino acids, soluble protein and proline*

In general, total free amino acids and proline contents decreased for all *Casuarina* spp. in unsterilized- than in sterilized soil, for all the tested frankiae (Fig. 5).

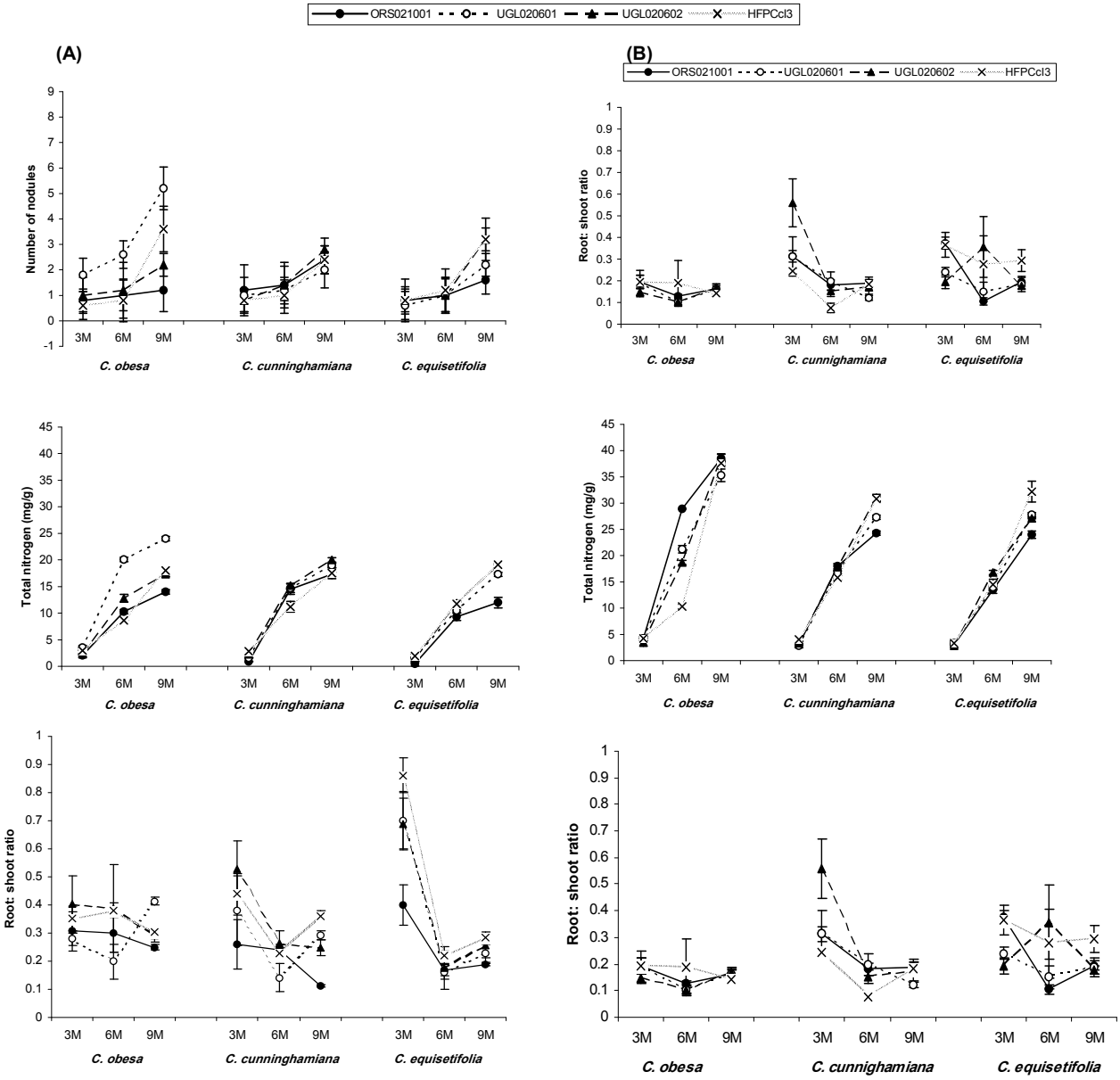


**Figure (3)**: Change in total dry mass (g), total nitrogen content (mg $g^{-1}$), and root: shoot ratios for different *Casuarina* spp. inoculated with different *Frankia* strains, after 3, 6 and 9 month in unsterilized soil (A) and in sterilized soil (B).

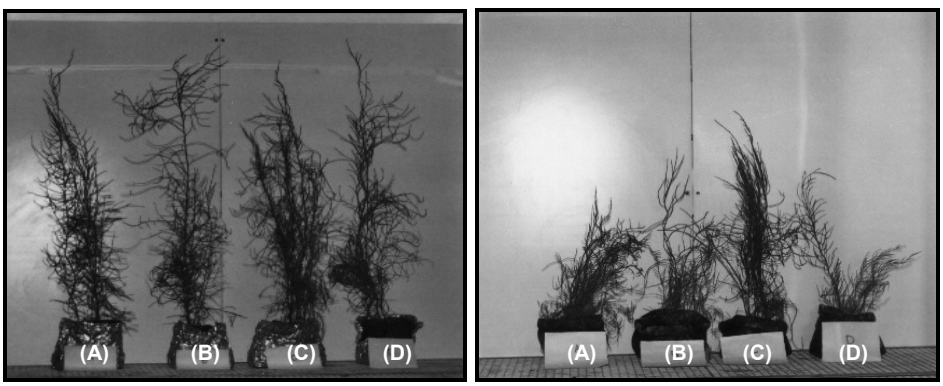


**Figure (4)**: Growth of *Casuarina obesa* plants, inoculated with different *Frankia* strains after 9 months in sterilized soil (right) and unsterilized soil (left).

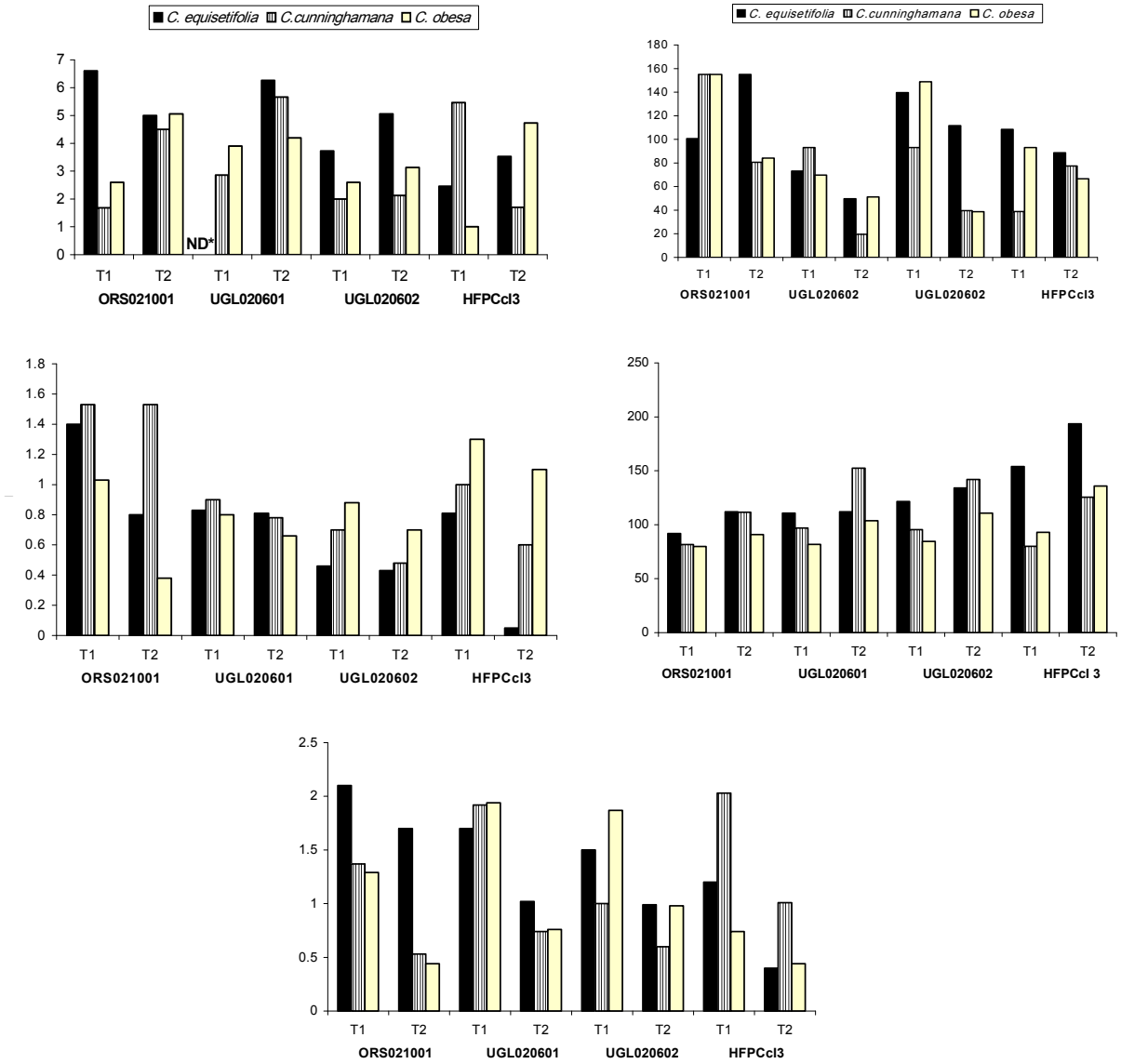


**Figure (5)**: Changes in catalase enzyme activity (unit $min^{-1}$ $gm^{-1}$ fresh matter), malondialdehyde content (nmol $gm^{-1}$ fresh matter), total free amino acids (mg $gm^{-1}$ dry matter), soluble protein content (mg $gm^{-1}$ dry matter) and praline content (mg/ $gm^{-1}$ dry matter) within different *Casuarina* spp. inoculated with different *Frankia* strains after 3 months. **T1**: in sterilized soil, **T2**: in unsterilized soil, **ND***, not determined.

In contrast, soluble protein content slightly increased for plants in unsterilized- than in the sterilized soil for all *Frankia* strains (data not shown). Only slight differences were found with the same casuarinas and frankiae combinations that was mentioned above.

## DISCUSSION

*Frankia* strains inhabit three distinct ecological niches, the root nodules, the rhizosphere, and the soil (Benson & Silvester, 1993). A considerable amount of information is available on *Frankia* strains isolated from root nodules and on their interaction with their host plants (Sayed, 1995; Maunuksela, 2001; Salem, 2003; Abd El-Karim, 2004). For the *Frankia*-actinorhizal plant symbiosis, the bacteria must maintain infective populations within the soil biotic community and establish themselves competitively in host rhizospheres. This process and its underlying mechanisms are not completely understood (Zimpfer *et al.,* 2002, 2003).

Soil microbes that are not diazotrophs may influence symbiotic N fixation either positively or negatively (Rojas *et al.,* 2002). Mycorrhizal fungi enhance symbiotic N fixation by improving host nutrition and perhaps water relations (Carling *et al.,* 1978; Barea & Azcon-Aguilar, 1983). However, answers are now being uncovered through the study of associations between plant roots and key members of the soil microbiota, mainly, bacteria, fungi and microfauna (Watkinson, 1998; Copley, 2000; Bradford *et al.,* 2002).

Most of the recent studies, on *Frankia* and its symbiosis with *Casuarina*, were carried out using pure *Frankia* cultures and plants grown in sterile soils, in

order to avoid contamination with other *Frankia* that may present naturally in soil (Zhang & Torrey, 1985; Sayed *et al.,* 2002a, b). More extensive field and laboratory investigations are required without soil sterilization where the biotic factors will interact naturally with *Frankia* and the processes of litter decomposition. This will allow more understanding of the above interactions. In agreement with this, Zimpfer *et al.* (2003) suggested a positive synergism between soil biota and *Frankia* inoculum with respect to *Casuarina cunninghamiana* infection.

Some 29 of the 68 species of the genus *Casuarina* are known to nodulate. *Frankia* sources from one *Casuarina* usually nodulate other *Casuarina* species. Large differences in the effectiveness of *Frankia*-*Casuarina* combinations occurred. Some frankiae are highly effective on *C. equisetifolia* but were ineffective on *C. cunninghamiana* and *vise versa* (Torrey, 1990; Sayed *et al.,* 1998). In soil, there are also infective and non- infective *Frankia* strains. (Baker, 1987).

In our study there were statistically significant synergistic interactions between soil biota and all the tested *Frankia* strains inoculated into unsterilized soil. Nodulation and plant performance were found two to three times greater, for plants in uninoculated, unsterilized soil (i.e. indigenous *Frankia* population) than plants inoculated with exogenous *Frankia* strains in sterilized and unsterilized soil (except for strain UGL020601 in unsterilized soil). These results suggest responses similar to those demonstrated in a complementary study (Sayed *et*. *al*. 2007). It is possible that elements added by the soil biota alter root morphology or induce other beneficial effects on plant growth and symbiosis. Coinciding with this, co-inoculation of *Burkholderia cepacia* and *Frankia* promoted root hair deformation and nodulation of alder seedlings, while inoculation with *Frankia* alone did not (Knowlton & Dawson, 1983).

At Dakar, Senegal, where no specific *Frankia* exists in soil, response of *C. equisetifolia* to inoculation with pure culture of *Frankia* strain ORS021001 showed an increase in plant biomass by 78% coupled with nitrogen fixation of the order of 3.07 ± 1.86g per tree during the 6 months following seedling transplantation (Gauthier *et al.,* 1985). In Zimbabwe, the height of *C. cunninghamiana* was increased by 50 - 70% over inoculated controls. It should be noted that all these experiments were done in nitrogen- deficient unsterilized natural field conditions (Subbarao & Rodreguez- Barrueco, 1995). In the current study, the synergistic effect between soil biota and *Frankia* is obvious from the differences in the results of plant performance in sterilized and unsterilized soil. This was evidenced by an increase in plant height (Fig. 4) and total nitrogen over 100% for plants in unsterilized soil than those in sterilized soil during 6 to 9 months following seedling transplantation and inoculation with different *Frankia* strains (Fig. 3). Shoot height, number of nodules, total plant dry mass and also total nitrogen increased with time in plants inoculated in unsterilized soil than those in sterilized soil (Fig. 1, 3). This effect may be due to either the presence of an intact soil biotic community that stimulates *Frankia* inoculum in the unsterilized soil or the exogenous *Frankia* stimulastes the indigenous *Frankia* population or both. Similar results were reported, in unsterilized soil, by Zimpfer *et al.* (2003). In the unsterilized soil, the introduced *Frankia* strains were unable to achieve nodulation as high as those of the indigenous *Frankia* populations. This failure might be due to the physiological status of the introduced *Frankia* lacking the capacity to nodulate *Casuarina* trees competitively in an unsterilized soil.

The physiological status of a specific *Frankia* population in soil could possibly be triggered by environmental factors such as host vegetation, litter decomposition rate, etc. In accordance with this proposed explanation, casuarinas might favor saprophytic growth of *Frankia* population and increase its competitive abilities with respect to root nodule formation (Hahn *et al.*, 1999; Maunuksela *et al.*, 1999). This possibility is also supported by those studies where only one population of *Frankia* was detected in nodules of the host plant, by *in situ* hybridization with different *Frankia* populations in soil (Zepp *et al.*, 1997a, b). The presence of actinorhizal plants (Benson & Silvester, 1993; Gauthier *et al*., 2000) or their close relatives (Smolander *et al*., 1990; Paschke & Dawson, 1992; Gauthier *et al*., 2000) in soils has increased nodulation capacities of soils suggesting that actinorhizal and some related plant species may release compounds into soil that favor *Frankia* growth, or increase nodulation capacity.

Girgis (1993) found that *Casuarina* seedlings would not nodulate axenically unless activated charcoal was added to the medium. Possibly the roots of the seedlings exude phenolics or other compounds which, unless adsorbed by charcoal, inhibit *Frankia* infection. The soil biotic community may act in a similar way to charcoal, either by sequestering or metabolizing compounds that inhibit nodulation. While steam sterilization for the soil does not generally release metabolites harmful to plants, there is a possibility that it releases compounds inhibitory to *Frankia* or nodulation of *Casuarina*. These compounds could account for the low nodulation capacity of sterilized soil inoculated with different *Frankia* strains.

In the present study, enzyme activities were denoted as indicators for plant performance concerning their variation under the current experimental conditions.

Catalase (CAT) is a heme-containing enzyme that catalyzes the dissolution of $H_2O_2$ into $H_2O$ and $O_2$ (Scandalias, 1990). The increase in CAT activity may provide plant protection from oxidative damage by rapid removal of $H_2O_2$ (Demiral & Türkan, 2004 a, b;

Chai *et al.*, 2005; Elkahoui *et al.*, 2005; Huang & Guo, 2005). In the current study, the activity of catalase enzyme increased markedly in all *Casuarina* spp. planted in unsterilized soil than those in sterilized soil, with the exception of *C. equisetifolia* and *C. cunninghamiana* when inoculated with *Frankia* strains ORS021001 and HFPCcI3, respectively. The higher CAT value indicates efficient $H_2O_2$ scavenging system in the plant and better protection against $H_2O_2$ (Scebba *et al.*, 1998). On the other hand, the decrease in CAT activity may result in $H_2O_2$ accumulation reacting with $O_2$ that can participate in diverse toxic oxidative reactions (Halliwell, 1982). The lower CAT activities with some strains indicate that the used *Frankia* strain is also involved indirectly in the process of plant protection.

The level of lipid peroxidation has been used as an indicator of cell membrane damage by free radicals under stress conditions (Elkahoui *et al.*, 2005). Malondialdehyde (MDA) is a product of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Halliwell & Gutteridge, 1989; McCord, 2000; Kukreja *et al.*, 2005). Our results indicate that MDA content decreased in plants in unsterilized- than those in sterilized soil. Only *C. equisetifolia* and *C. cunninghamiana*, inoculated with *Frankia* strains ORS021001 and HFPCcI3, respectively, recorded an increase in the MDA activity. The lower MDA content in both *C. obesa* and *C. cunninghamiana* in unsterilized soil indicates that these plants are more tolerant to oxidative stress under these conditions. It can be concluded also that CAT and MDA levels are closely related to the sensetivity of plant species to soil factors that interact with plant performance.

The decreased soluble protein content of plants, in sterilized soil, was accompanied with a pronounced accumulation of free amino acids in leaves, indicating that it might be due to protein hydrolysis into amino acids which might participate in osmotic adjustment under stress (Evers *et al.*, 1997). On the contrary, better plant performance was obtained in unsterilized soil (Fig. 5), indicating that these plants are not exposed to osmotic stress.

Thomas *et al.* (1992) reported that the high concentrations of amino acids are observed in many plants and could result from catabolic processes that accompany a decrease in plant growth rate. Similarly, other workers found that protein hydrolysis occurring in plants was always associated with varied increments in proline contents (Irigoyen *et al.*, 1992). Our results coincide with the previously mentioned ones on that total free amino acids decreased when all *Casuarina* plants were planted in unsterilized soil, and inoculated with different *Frankia* strains, than in sterilized soil. In addition, soluble protein content (i.e. synthesis) increased, in unsterilized soil, with all *Frankia* strains (data not shown).

Proline can serve as a protector of enzyme denaturation (Paleg *et al.*, 1984), a reservoir of nitrogen and carbon (Fukutaku & Yamada, 1984) or as a stabilizer of the machinery for protein synthesis (Kandpal & Appaji- Rao, 1985). Rapid accumulation of proline in plant tissue is a direct consequence of salinity stress (Lutts *et al.*, 1996). We found that the content of proline decreased in all *Casuarina* spp, in unsterilized soil, than in sterilized soil. These results and the previous data on nodulation and plant performance criteria indicate that plants in sterilized soil are lacking growth stimulating factors that are found only in unsterilized soil and that these plants are somehow considered under stress compared to those plants with indigenous soil microbiota (i.e. unsterilized soil).

Soil microbiota populations are immersed in a framework of interactions known to affect plant fitness and soil quality. There are much research addressed at understanding the diversity and dynamics of rhizosphere microbial populations and their co- operative activities (Barea *et al.*, 2005). The general rhizosphere effect of these organisms could help the plant by maintaining the recycling of nutrients, through the production of hormones, providing resistance to microbial diseases and tolerance to toxic compounds (Morgan *et al.*, 2005). As mentioned before, nodulation was significantly low even after inoculation with effective *Frankia*. In other words, indigenous frankiae performed better, in symbiosis with the tested casuarinas, than exotic frankiae except in some cases as shown above. Similar results were also reported by Rajendran & Devaraj (2004). Our findings suggest the possibility that specific members of the rhizosphere biotic community may interact with *Frankia* and *Casuarina* to increase nodulation and plant growth.

Generally, the presence of the native soil microbiota in inoculated and uninoculated, unsterilized soil increased plant nodulation and growth responses than in sterilized soil. It is concluded that, in most cases, the natural microbiota in the host rhizosphere is adapted for developing sufficient *Casuarina* productivity. It is only recommended to use exotic frankiae where soils lack the presence of an effective strain.

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## المحتوى الميكروبى الحيوى الملاصق للجذور والعلاقة التكافلية بين الفرانكيا والكازوارينا

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## الملخص العربـــى

تم استخدام أربعة سلالات من الفرانكيا فى تلقيح أربعة أنواع من الكازوارينا فى تربة معقمة وغير معقمة. وقد تم قياس أداء النبات بالنسبة للنمو والنشاط الإنزيمي بعد ثلاثة أشهر من التلقيح. تم تقدير إنزيمات الكاتاليز (CAT) وإنزيمات أكسدة الدهون (MDA) والبروتينات الكلية والأحماض الأمينية الحرة والبرولين وذلك للنباتات المعاملة بعد ثلاثة أشهر من التلقيح. أما تكوين العقد الجذرية ومؤشرات أداء النبات (النمو) فقد تم تقديرها بعد ثلاثة وستة وتسعة أشهر من التلقيح. وقد كان تدرج أداء النبات بالنسبة للمعاملات المختلفة تنازليا كالتالى: النباتات الغير ملقحة فى التربة الغير معقمة ≤ النباتات الملقحة فى التربة الغير المعقمة > النباتات الملقحة فى التربة المعقمة.

إن تجمعات الفرانكيا الأصلية الموجودة فى التربة أكثر تكيفا- للاستفادة من المحتوى الحيوى الموجود فى التربة الغير معقمة- من سلالات الفرانكيا المدخلة إلى التربة ماعدا فى حالات قليلة.إن الزيادة فى إنزيم الكاتاليز والنقص فى أكسدة الدهون (مالون داى ألدهيد) والبرولين (فى النباتات بالتربة الغير معقمة) يدل على أن الفرانكيا الأصيلة بالتربة -بجانب الميكروبات المصاحبة لها- لها فائدة أكثر بالنسبة لأداء النبات بوجه عام. والخلاصة فان المجتمع الحيوى للتربة يمكن أن ينتج العوامل الملائمة المطلوبة لحماية النبات ولظروف نموه الأمثل وذلك جنبا إلى جنب مع سلالات الفرانكيا ذات الكفاءة فى تكوين العقد الجذرية وتثبيت النيتروجين.