Soil Biol. Biochem. Vol. 22, No. 1, pp. 57–64, 1990
Printed in Great Britain

0038-0717/90 $3.00 + 0.00
Pergamon Press plc

COMPETITION FOR NODULATION AND $^{15}N_2$-FIXATION BETWEEN A Sp^+ AND A Sp^- *FRANKIA* STRAIN IN *ALNUS INCANA*

F. Kurdali, G. Rinaudo, A. Moiroud and A. M. Domenach
Université Lyon 1, I.A.S.B.S.E., Laboratoire de Microbiologie des Sols, U.R.A. C.N.R.S. 71, 43, boulevard du 11 Novembre 1918, 69622-Villeurbanne Cédex, France

(*Accepted* 16 *June* 1989)

Summary—The behaviour of a Sp^+ and a Sp^- strain of *Frankia* for nodulation and N_2-fixation in *Alnus incana* with pure and mixed inocula was investigated under greenhouse conditions, using spore formation as a morphological marker for strain recognition. The results showed that, in an artificial medium, both strains coexisted on the same root system, but the Sp^- strain exhibited greater specific activity (N_2-fixation) that the Sp^+ strain. When a Sp^- strain was introduced into soil containing indigenous Sp^+ *Frankia*, a low proportion of nodules were formed by the new strain and exhibited lower specific activity than Sp^+ nodules. It is evident that competitive ability varies with strains and that effectiveness is affected even if nodulated plants are transplanted into soil. Some soil factors possibly negatively affecting the persistence of introduced Sp^- *Frankia* strain are discussed.

INTRODUCTION

Alnus incana is an important actinorhizal plant found in the mountains of the southern and eastern part of France, and is able to form root nodules in symbiosis with *Frankia* and to grow in poor calcareous soils. This alder species is also used in soil reclamation because it improves soil nitrogen through N_2-fixation and turnover of N-rich litter (Danière *et al.*, 1986; A. M. Domenach and F. Kurdali, in press).

In natural conditions, alder trees are usually well nodulated indicating that the root nodule endophyte is widely distributed.

To increase the symbiotic effectiveness of alder by introducing a more efficient strain of *Frankia*, it is necessary to know the ability of this strain to compete for nodulation with the native strains, and also if it can coexist on the same root system (Reddel and Bowen, 1985; Benson and Hanna, 1983).

The study of competition between *Frankia* strains is very important for a full understanding of the biology of actinorhizae, and the improvement of actinorhizal symbioses. In the case of *Rhizobium*–legume symbioses, many papers have been published concerning the competition between *Rhizobium* strains under both laboratory and field conditions (Amarger, 1981; McLoughlin and Dunican, 1985; Moawad *et al.*, 1988).

Competition for nodulation among *Rhizobium* strains can be studied by using techniques related to the serological characters, the intrinsic antibiotic resistance profiles and the genetic diversity of the strains (Amarger and Lobreau, 1982; McLoughlin *et al.*, 1985; Clayet-Marel and Crozat, 1982; Skradeta, 1973). In contrast, very little is known about the persistence of *Frankia* strains in the soil or the competition for nodulation of actinorhizal plants. The main limiting factor for conducting such studies until now, has been the unavailability of reliable techniques to follow a marked strain in its natural environment: few spontaneous antibiotic mutants have been isolated (Normand and Lalonde, 1986) and serology appeared to be not sensitive enough to demonstrate strain differences (Simonet, 1983). Nowadays, researchers point out that applications of molecular biology would probably allow further investigations in order to discriminate between *Frankia* strains.

However, two distinct types of *Frankia* strains can be easily recognized: Sp^+ for nodules containing sporulating endophyte and Sp^- for non-sporulating ones (Van Dijk, 1978; Van Dijk and Merkus, 1976). The two types of nodule differ in several important aspects (Tjepkema *et al.*, 1986). Sp^+ strains are more infective than Sp^- strains (Houwers and Akkermans, 1981; Van Dijk, 1984). However, Sp^+ nodules are probably less effective in supporting growth of the host plant than Sp^- nodules (Hall *et al.*, 1979; Normand and Lalonde, 1982; Simon *et al.*, 1985), and may have lower nitrogenase activity (VandenBosch and Torrey, 1984; Wheeler *et al.*, 1986).

Since the ability to produce spores within the nodules is demonstrated to be under genetic control (Van Dijk, 1978; VandenBosch and Torrey, 1985) the presence of spores may be used as a mean to distinguish between competing strains (Van Dijk, 1984; Houwers and Akkermans, 1981; Kurdali *et al.*, 1989a). But, this method cannot demonstrate whether the two strains coexist in the same nodule.

We report competition between two *Frankia* strains: a Sp^+ as an indigenous strain and a Sp^- as a new strain introduced for nodule formation and N_2-fixation efficiency on *A. incana* grown in both artificial substrate and soil under greenhouse conditions. Indeed, at Ornon site (Danière *et al.*, 1986), *A. incana* always forms nodules of a Sp^+ type which is characterized by the presence of a great number of sporangia. However the actual endophyte is not able to form effective nodules on *Alnus glutinosa* (Kurdali *et al.*, 1989a). In the same way, no nodules have been found on *A. glutinosa* from soils where *A. incana* grows on site. Therefore, these particular characters will be used in studies concerning the introduction of a new strain into soil containing indigenous *Frankia*, and to control its survival under the new conditions. A first experiment was conducted on artificial substrate in order to determine the genetically-determined competitive ability of *Frankia* strain for nodulation, while the second and third experiments were performed in soil to show if the new introduced strain can compete with indigenous *Frankia* and survive despite the biotic and abiotic factors.

MATERIALS AND METHODS

Plant materials

Seeds of *A. incana* (L.) Moench were collected from trees situated at 1450 m near "col d'ornon", Isere, France. Seeds were surface sterilized for 10 min with hydrogen peroxide (30% v/v) and rinsed with distilled water on moistened filter paper. At the cotelydon stage seedlings were transplanted into pots containing either an artificial substrate (experiment 1) or soil (experiment 2).

Inocula preparation

Sp^+ inoculum: Sp^+ nodules were collected from the same *A. incana* stand from where seeds were obtained. A sample of 2 g of nodules were crushed in a mortar in the presence of 3% (w/v) P.V.P (polyvinyl pyrolidone) in distilled water, filtered on a 100 μm sieve and diluted with 100 ml of sterile water. Such a procedure was used because no Sp^+ endophyte was available in pure culture.

Sp^- inoculum: since the Sp^+ inoculum was obtained from crushed nodules, it was necessary to use the same inoculation procedure for the Sp^- endophyte. To that purpose seedlings of *A. incana* were inoculated some months before the experiment with the Sp^- *Frankia* strain (Al 15). This strain had been isolated from *A. incana* root nodules and cultured in 500 ml of F.T.W medium (Simonet *et al.*, 1985). The colonies were then homogenized by repeated vigorous passages through a narrow gauge needle (0.8 mm) and diluted with 100 ml of sterile water. A sample of 2 g of nodules (fresh weight) were collected 5 months after inoculation and crushed as described for Sp^+ nodule homogenate preparation.

Inoculation procedure

Experiment 1 (artificial substrate): 50 seedlings were transplanted into pots containing dehydrated clay as an inert artificial substrate (Allsmeer, The Netherlands) and supplied once a week with N-free Crone's mineral nutrient solution. Seedlings were then subdivided into 5 sets (10 seedlings for treatment) and inoculated with *Frankia* as follows:

- Set 1: Control treatment, seedlings were inoculated with Sp^+ nodule homogenate (indigenous *Frankia* strains);
- Set 2: Control treatment, seedlings were inoculated with Sp^- nodule homogenate (new strain);
- Set 3: Seedlings were inoculated with a mixture of Sp^+ and Sp^- nodule homogenates in equal proportion. This set was made in order to study the competitive ability between the two types of *Frankia* for nodulation;
- Set 4: Seedlings were first inoculated with the Sp^+ *Frankia* endophyte and then, 2 months later, inoculated with the Sp^- strain;
- Set 5: Seedlings were first inoculated with the Sp^- strain and then, 2 months later inoculated with the Sp^+ endophyte. The purpose of sets 4 and 5 was to evaluate the possibility to introduce a new *Frankia* strain on nodulated plants.

Experiment 2 (soil containing Sp^+ indigenous Frankia *endophytes):* Soil samples were obtained from the surface layer (10 cm) on the site where Sp^+ nodules were collected. The main characters of the soil were: pH = 8.3, $CaCO_3 = 63.8\%$, C-organic = 0.5–1.3%, N-organic = 0.06%, $N\text{–}NH_4^+ = 0$, $N\text{–}NO_3^- = 0$ (Danière *et al.*, 1986). Seedlings were transplanted in pots previously filled with soil. Four sets were set up as follows:

- Set A: Control treatment; seedlings were transplanted into soil without addition of pure *Frankia* strain;
- Set B: Before transplanting, 5 ml of a suspension of pure culture of the Sp^- strain (Al 15) were added into each pot. This quantity is sufficient to produce approximately the same nodule biomass which can be obtained by inoculation with a Sp^+ nodule homogenate (2 g of nodule fresh weight per 100 ml H_2O) (Domenach *et al.*, 1988; Kurdali *et al.*, 1989a). This set was performed in order to study the competitiveness of the Sp^- strain for nodule formation versus the indigenous Sp^+ *Frankia* strains.
- Set C: Seedlings were inoculated with 5 ml of a suspension of the Sp^- *Frankia* strain 2 months after transplanting into soil. This set was to obtain evidence that new nodules are formed when a new strain of *Frankia* is introduced on sites where plants are already nodulated with indigenous strains.
- Set D: Seedlings were transplanted into artificial substrate and inoculated with the Sp^- strain. Two months later, plants were transplanted into soil. The purpose of this set was to study the possibility to introduce a new *Frankia* strain by transplanting nodulated alder into soil containing indigenous *Frankia* strains.

Experiment 3 (survival of Sp^- strain introduced into soil): this experiment followed the second one. Its aim was to show if the new strain introduced can survive in the soil used. The soil used in this study did not contain Sp^- strains which might induce nodule formation on *A. glutinosa*. On the other hand, Sp^+ strains were not able to form effective nodules on this plant species (Kurdali *et al.*, 1989a). While Sp− strain (Al 15) was able to form effective nodules (Domenach *et al.*, 1988). If Sp− strain introduced in set B and C survives in the soil, it can infect *A. glutinosa* and form effective nodules. Thus, these data lead us to use the soil of set B and C to transplant *A. glutinosa* seedlings and to measure the nodulation after 3 months growth.

Plant harvest

Height of the plants were recorded 157 days after inoculation. During this period, a positive correlation occurred between height and biomass of plants grown on N-free medium (C. Danière, personal communication). To verify if the nodules formed by the new introduced strain can persist and fix N_2 effectively over a prolonged period, all plants were harvested 460 days (15 months) after the first inoculation.

N_2-fixation

The labelling process was made on nodules attached to root fractions to avoid damages. They were then carefully placed in a 150 ml flask which served as an incubation chamber. Air was then evacuated from the flasks and replaced by a gas mixture containing 50 ml of N_2 enriched with 99 atom% $^{15}N_2$ and 12.5 ml of O_2. The gas pressure inside the flasks was equilibrated at the atmospheric pressure with air via a double needle. $^{15}N_2$ final concentration was 37% of total N_2 in the flasks as measured by optical emission spectrometry (Sopra GS1, Bois-Collombes, France). Flasks were kept in the dark at 25°C for 1 h. Nodules were then separated into the two types (Sp+ and Sp−) by sections observed under a light microscope. Dry weight and total nitrogen (Bremner, 1965) of nodules and root fractions were determined on each sample. ^{15}N enrichment was measured on the whole lot (nodules + root fractions) by methods described by Domenach and Chalamet (1977) using mass spectrometry (VG SIRA 12, Manchester, U.K.).

Microscopic examinations

To distinguish between Sp+ and Sp− nodule types, 2–3 lobes of each nodule were examined under light microscopy. For each set, *ca* 100 longitudinal hand sections of nodule lobes were stained with Cotton Blue in lactic acid for 1 min and observed under the microscope (Kurdali *et al.*, 1989a). Nodules were then separated into the two types. Sp+ nodules were easily recognizable by the presence of a great number of sporangia which are at some distance from the meristem. In that case one section showing sporangia was enough to classify the nodule as Sp+ type.

RESULTS

Plant growth

Experiment 1: the aim of the experiment was to evaluate the competition between the Sp+ and Sp− *Frankia* strains.

The comparison of the plant height at 0.05 level (Fig. 1) showed no significant differences in growth rates among sets. Likewise, the introduction of a new strain (set 4 and 5) did not modify significantly the dry matter of shoots and roots, and the N-content of shoots (Table 1). These data did not indicate if the two nodule types coexisted or if one of them dominated.

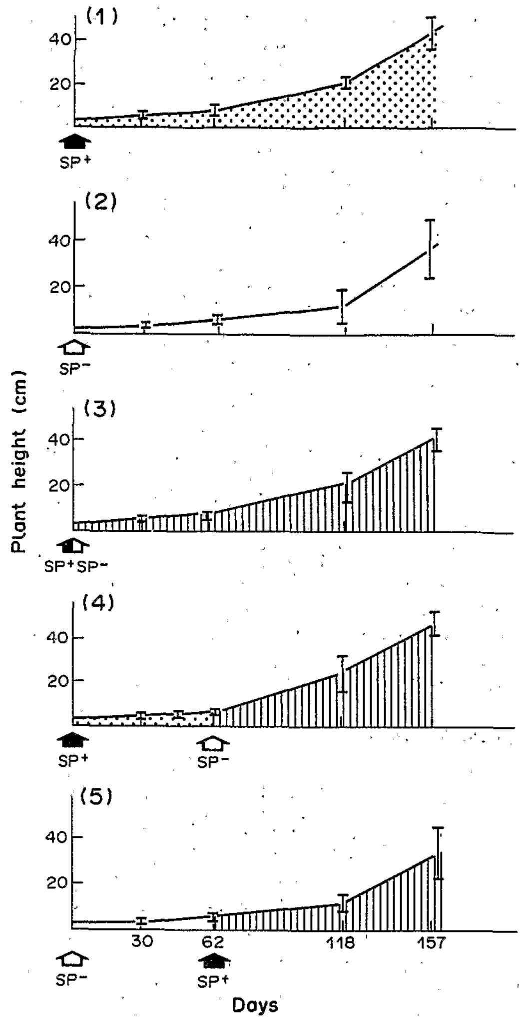

Fig. 1. Kinetics of growth of *A. incana* grown on artificial medium and inoculated with Sp+ or Sp− strains of *Frankia*, $n = 10$.

Experiment 2: This experiment was carried out in order to observe soil effects upon the introduction of a new *Frankia* strain.

Table 1. Dry weight and N-content of shoots and roots of *A. incana* grown on both artificial medium (experiment 1) and soil (experiment 2) and inoculated with Sp+ or Sp− strains of *Frankia*

Set	Shoot dry wt (g plant^{-1})	Root dry wt (g plant^{-1})	Shoot N-content (mg plant^{-1})
Experiment 1			
1	9.8 (1.4)*	6 (3)	209 (32)
2	9.6 (2.5)	4 (3)	206 (40)
3	9.2 (1.4)	6 (2)	202 (31)
4	10 (1.8)	5 (1)	221 (40)
5	12 (3.0)	7 (3)	270 (60)
Experiment 2			
A	0.4 (1.0)	0.5 (0.3)	8 (3)
B	0.5 (0.2)	0.6 (0.2)	11 (4)
C	0.5 (0.1)	0.6 (0.3)	11 (4)
D	1.5 (0.4)	1.4 (0.3)	32 (9)

*SD, $n = 10$.
See Materials and Methods for explanation of treatments (sets).

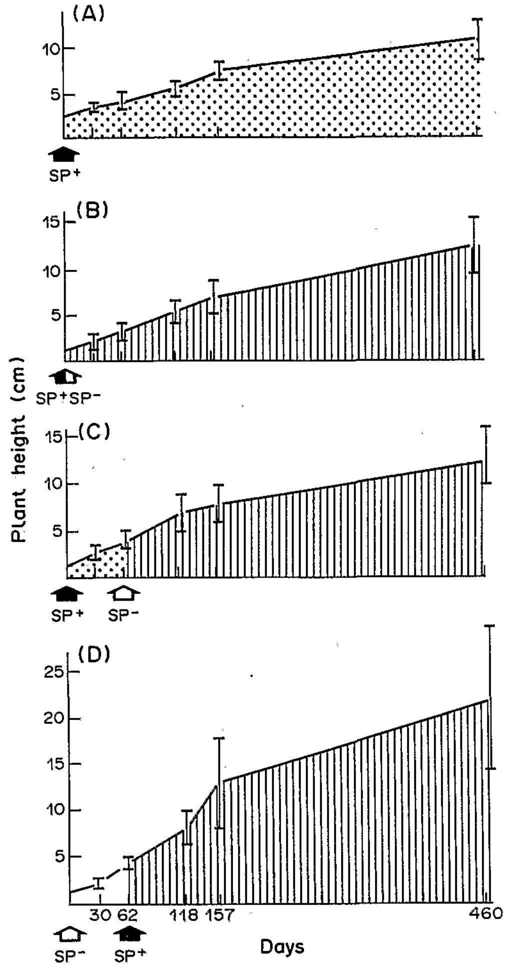

Fig. 2. Kinetics of growth of *A. incana* grown in soil containing Sp^+ indigenous *Frankia* endophyte and inoculated with Sp^- strains of *Frankia*, $n = 10$.

The rate of plant growth was clearly lower in this experiment than in the first one (Fig. 2). The comparison between plant heights showed that the growth rate of sets A, B and C respectively did not differ significantly. In contrast, that of set D was significantly higher.

The superiority of set D was also observed in the dry weight and N-content of shoot and root respectively determined at the end of the experiment (Table 1).

Competition for nodule formation

Experiment 1: dry matter of the two nodule types was used as a measure to determine the competitiveness of Sp^+ and Sp^- *Frankia*. No significant difference was observed in total nodule dry weight between the sets studied (Fig. 3), but the proportion of Sp^+ and Sp^- nodules varied widely in sets 3, 4 and 5.

When the two strains were introduced simultaneously (set 3), Sp^+ was 10 times greater than Sp^- nodule dry weight (0–3 Sp^- nodules). Sp^+ type represented 92% of the total nodule biomass.

The introduction of the Sp^- strain to plants already nodulated with the Sp^+ indigenous endophyte (set 4) produced a second round of infection. The new strain formed 17% of the total nodule dry weight at the end of the experiment.

In set 5, with plants already nodulated by the Sp^- strain, the Sp^+ strain produced a second infection which represented 28% of total nodule dry weight.

Experiment 2: the dry weights of Sp^+ and Sp^- nodules are given in Fig. 4 and their relative proportions in Fig. 5.

For sets B and C, the introduction of the Sp^- strain (pure culture) into soil containing the indigenous Sp^+ *Frankia* endophyte did not lead to a significant increase in nodule number, the inoculum being introduced at the beginning of the experiment (set B) or 2 months later (set C). Mean dry weight of Sp^- nodules represented only 5–7% of the total nodule biomass including plants which did not bear Sp^- nodules (2 plants in set B and 2 in set C). In set D, the transplanting of plants bearing Sp^- nodules into soil containing Sp^+ indigenous *Frankia* strains resulted in a higher growth rate. But, surprisingly, at the end of the experiment, the Sp^- nodules represented only 21% of the total nodule biomass, 79% of the nodules originating from the second infection by the Sp^+ indigenous *Frankia* strains.

$^{15}N_2$-fixation

Experiment 1: the efficiency of the symbiosis was evaluated by the quantity of N_2 fixed g^{-1} nodule dry weight h^{-1} (Table 2). For control plants, it can be observed that the specific activity of Sp^- nodules was 2–3 times as much as Sp^+ nodules. Moreover, the

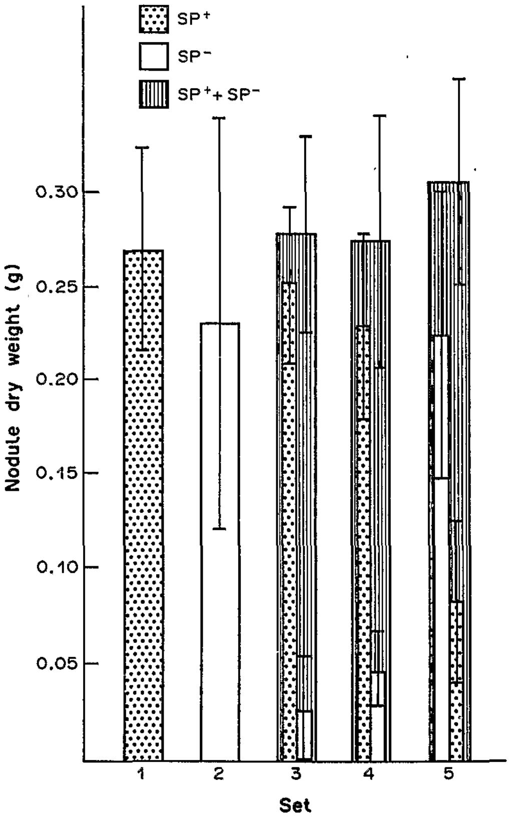

Fig. 3. Nodule biomass of *A. incana* grown on artificial medium and inoculated with Sp^+ or Sp^- strains of *Frankia*, 15 months after the first inoculation, $n = 5$.

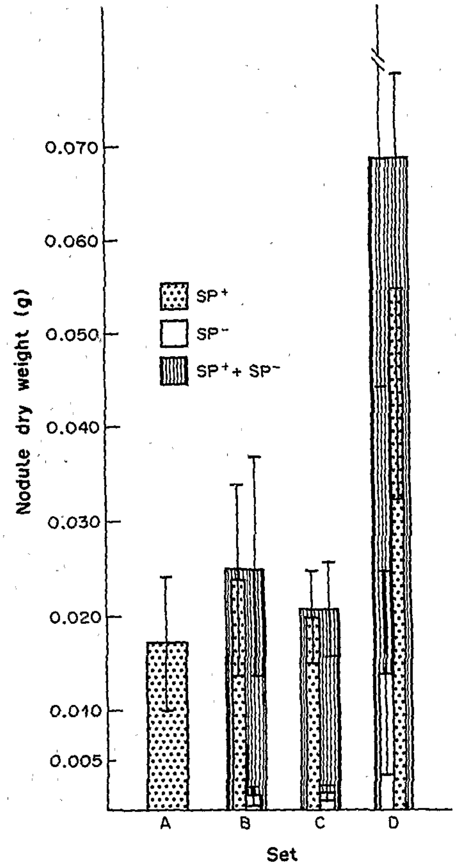

Fig. 4. Nodule biomass of *A. incana* grown in soil containing Sp^+ indigenous *Frankia* endophyte and inoculated with Sp^- strains of *Frankia*, 15 months after the first inoculation, $n = 6$.

higher specific activity of the Sp^- is reflected in all the sets studied.

Experiment 2: Unlike the first experiment, the specific activity of Sp^+ nodules was higher than that of Sp^- nodules (Table 3). Sp^- nodules from sets B or C were combined to allow ^{15}N measurements since Sp^- nodule type yield from each repetition was not sufficient for a separate analysis. In set D the nitrogen fixing activity of Sp^- nodules was lower than that of Sp^+, which indicates that, after transplanting, the activity of Sp^- nodules had decreased significantly.

As regard to experiment 3, no Sp^- nodules were found on *A. glutinosa* planted in soil obtained from sets B and C which indicates that Sp^- strain did not maintain its viability in this soil.

DISCUSSION

The sporulating capacity in host nodules is considered to be a genetically-stable characteristic of the endophyte not influenced by the host plant (Van Dijk, 1978; VandenBosch and Torrey, 1985). Thus, the presence of sporangia may be used as a means to distinguish between competing strains (Van Dijk, 1978; Kurdali *et al.*, 1989a).

Several authors reported that Sp^- nodules are more effective in supporting plant growth than Sp^+ nodules (Hall *et al.*, 1979; Normand and Lalonde, 1982; Simon *et al.*, 1985; VandenBosch and Torrey, 1984; Wheeler *et al.*, 1986).

In the first experiment with N-free medium, the growth rate of *A. incana* inoculated either with Sp^+ or Sp^- strain did not differ significantly. Moreover, there was a slight difference in biomass and N-content between Sp^+ and Sp^- nodules, indicating that their infective capacity was comparable. This result agrees with that obtained by Simonet (1983) who demonstrated that the proportion of spores germinated *in vitro* was negligible. However, the specific activity (N_2-fixation) of Sp^- nodules was 2–3 times greater than with Sp^+ nodules. This would be possibly related to a variation of the rate of N_2-fixation with time: the initial development of Sp^- nodules would be delayed as compared with Sp^+ nodules (Kurdali *et al.*, 1989a). Likewise, Wheeler *et al.*, (1986) found that seedlings of *Alnus rubra* inoculated with Sp^- isolates fixed three times more nitrogen than those inoculated with Sp^+ nodule homogenate.

The inoculation of plants with a mixture of Sp^+ and Sp^- nodule homogenates in equal proportion showed that the Sp^+ endophyte was more competitive than the Sp^- one for nodulation. In contrast, the specific activity of N_2-fixation of the Sp^- nodules was higher than that of the Sp^+ ones.

The results of the second experiment obtained with plants grown in soil also showed that Sp^+ nodules were more dominant than Sp^- ones (set B). This could indicate that, the infective particles were spores as suggested by several authors (Akkermans and Van Dijk, 1976; Houwers and Akkermans, 1981; Van Dijk, 1984) who found that Sp^+ nodules were 100–1000 times more infective on *A. glutinosa* than Sp^- nodules. Moreover, in soil, Sp^+ nodules fixed N_2 more actively than the Sp^- nodules, although with artificial substrate, the Sp^- ones was more active, which indicated that the Sp^- strain was affected by soil factors.

When the Sp^- strain was introduced to plants already bearing Sp^+ nodules, the new strain induced a second round of infection in artificial media (set 4). Likewise, in a previous experiment, we found that a second inoculation produced new effective nodules on both *A. incana* and *A. glutinosa* (Kurdali *et al.*, 1989a). Since the ability for nodulation depends on inoculum concentration of the new strain introduced (Houwers and Akkermans, 1981) it seems possible to introduce a new *Frankia* strain into a soil already containing an indigenous endophyte. However, results from set C demonstrate that, despite the introduction of a sufficient concentration of Sp^- strain (pure culture) on nodulated *A. incana*, only 5% of the total nodule biomass was found to be Sp^- type. Moreover, the nodules thus formed had less specific activity than Sp^+ nodules for N_2-fixation.

A method to introduce a *Frankia* strain in a soil ecosystem is by introducing nodulated alder plants bearing that strain into a soil free of *Frankia* (Arveby and Huss-Danell, 1988). In this case it was shown that *Frankia* is able to survive and to remain effective, since there is no competition with an indigenous *Frankia* flora. In our second experiment, the introduction of Sp^- *Frankia* by a nodulated *A. incana* into soil containing an indigenous *Frankia* strain (set D) resulted in an increase of plant growth for a few

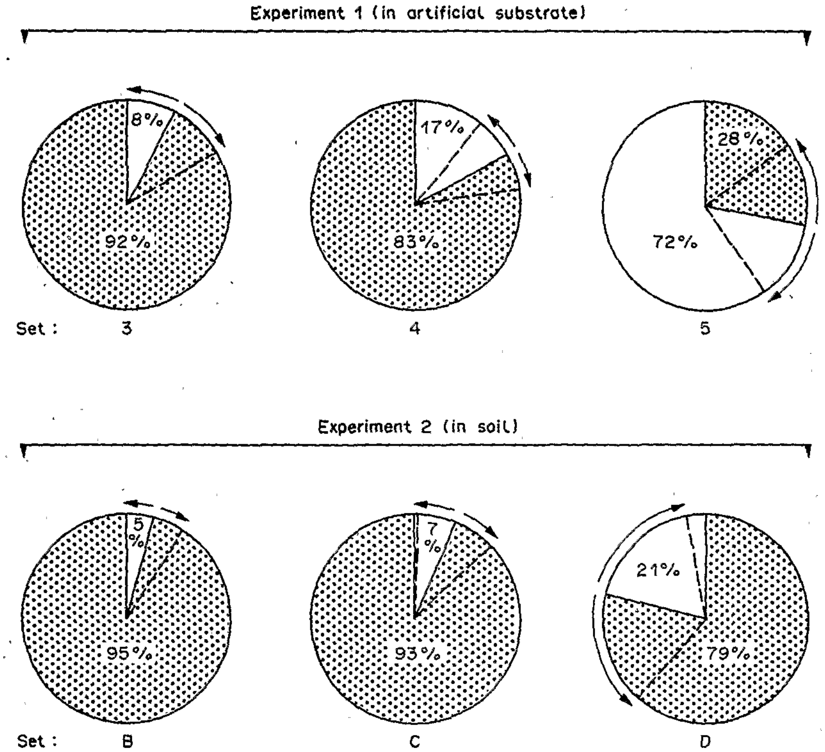

Fig. 5. Proportion of Sp^+ and Sp^- nodule biomass of *A. incana* grown in both artificial substrate and in soil containing Sp^+ indigenous *Frankia* endophyte and inoculated with Sp^+ or Sp^- strains of *Frankia*. Shaded area: Sp^+, white area: Sp^-. Arrows indicate SD.

Table 2. N-content in nodules (Sp^+ and Sp^- types) and root fractions, atom $\%^{15}N$ in excess and N-uptake by plant and per 1 g nodule dry weight after a 1 h labelling period for plants grown on artificial substrate

Set	Spore	N-content (mg pl^{-1}) Nodules	Roots	$\%^{15}N$ excess nod. + roots	Amount of ^{15}N (μg) nod. + roots	N_2-fixed ($\mu g\ h^{-1}$) ($pl.^{-1}$)	(g nod $d \cdot w^{-1}$)
1	+	3.8 (0.6)*	3.4 (1.0)	0.066 (0.016)	4.7 (2.0)	13 (10)	50
2	−	2.9 (0.3)	2.7 (2.0)	0.217 (0.118)	12.2 (3.0)	33 (17)	143
3	+	3.9 (1.1)	4.3 (1.4)	0.096 (0.017)	7.9 (0.8)	21 (5)	84
	−	0.4 (0.3)	0.8 (0.8)	0.213 (0.171)	2.6 (1.0)	7 (5)	225
4	+	3.3 (0.8)	3.5 (0.8)	0.112 (0.064)	7.6 (4.0)	21 (23)	92
	−	0.6 (0.2)	0.8 (0.8)	0.159 (0.142)	2.3 (1.5)	6.2 (4)	138
5	+	1.1 (0.4)	1.8 (1.0)	0.062 (0.025)	1.7 (0.8)	4.8 (4)	57
	−	3.1 (0.9)	4.9 (4.0)	0.084 (0.027)	6.6 (3.0)	18 (13)	82

*SD, $n = 5$.

Table 3. N-content in nodules (Sp^+ and Sp^- types) and root fractions, atom $\%^{15}N$ in excess and N-uptake by plant and per g nodule dry weight after a 1 h labelling period for plants grown on soil containing Sp^+ indigenous *Frankia* endophyte

Set	Spore	N-content (mg pl^{-1}) Nodules	Roots	$\%^{15}N$ excess nod + roots	Amount of ^{15}N (μg) nod + roots	N_2-fixed ($\mu g\ h^{-1}$) ($pl.^{-1}$)	(g nod $d \cdot w^{-1}$)
A	+	0.25 (0.1)*	0.13 (0.1)	0.268 (0.024)	1 (0.4)	2.7 (1.3)	159
B	+	0.30 (0.1)	0.3 (0.2)	0.110 (0.047)	0.66 (0.16)	1.8 (0.6)	78
	−	0.03 (0.01)	0	0.1039	0.0312	0.085	27
C	+	0.27 (0.03)	0.086 (0.03)	0.188 (0.11)	0.67 (0.25)	1.8 (0.7)	91
	−	0.03 (0.01)	0	0.104	0.0312	0.081	30
D	+	0.6 (0.2)	0.33 (0.17)	0.198 (0.076)	1.84 (0.6)	4.9 (1.7)	89
	−	0.1 (0.09)	0.08 (0.05)	0.142 (0.068)	0.25 (0.1)	0.67 (0.28)	48

*SD, $n = 6$, but in set B or C, 2 plants did not bear Sp^- nodules and the calculation was made on 4 plants with Sp^- nodules.

months (Fig. 2) though the new strain lost its activity eventually. Thus, the Sp^- nodules were less active 400 days after transplanting, when they began to show darker zones. Moreover, a large proportion of nodules appeared to be Sp^+ type (80% of total nodule dry weight) and their specific activity was greater than the initial nodules (Sp^-). These results seem to be contradictory if compared with those from set 5 of the first experiment: Sp^- nodules persisted and fixed more nitrogen than Sp^+ nodules. Thus, it becomes evident that the competitive ability varies with strains and is affected by the soil.

The results obtained by Akkermans and Van Dijk (1976), Quispel (1955) and Van Dijk (1979, 1984) concerning the persistence of nodulation capacity of nodule homogenates introduced into soil, were inconsistent. Smolander *et al.* (1988) concluded that the actual variability observed may be due either to heterogeneity of nodule material or to the effect of soil characteristics on survival of *Frankia*. Also, Houwers and Akkermans (1981) concluded that growth of *Frankia* may differ according to soil type.

Results obtained from the third experiment show that the Sp^- strain did not survive in the soil, indicating that soil factors could affect the maintainance of *Frankia* strains.

The actual soil had a $pH = 8.3$ and $CaCO_3 = 63\%$, that might negatively affect the persistence of the new *Frankia* strains introduced, since the introduction of Sp^- strain via nodulated plants led to the production of a second round of infection and allowed the formation of effective nodules when an artificial substrate was used. In this context some authors have reported a positive correlation between the pH and nodulation capacity in alder (Smolander and Sundman, 1987). Thus, a low pH caused an inhibitory effect on nodulation (Wheeler *et al*; 1981). Also, a very alkaline soil with a high $CaCO_3$ content would be a detrimental factor for maintainance of certain *Frankia* strains.

In a field experiment, a ($AGN_{24}D$) *Frankia* strain was introduced via nodulated *A. incana* into the same soil. The nodules persisted there over 2 years and increased in volume but not in number (A. Moiroud and F. Kurdali, unpublished results). It thus appears that *Frankia* may interact differently with certain factors such as the pH according to strains (Faure-Raynaud *et al.*, 1986; Burggraaff and Shipton, 1982). Likewise, the growth of two *Frankia* strains isolated from *A. incana* nodules in pure culture was low to pH lower than 4 and higher than 7 (Smolander *et al.*, 1988).

Despite the fact that Sp^- strains have certain advantages over Sp^+ ones such as their possible ability to grow saprophitically (Weber, 1986), Sp^+ strains seem to be more tolerant of soil conditions. *A. incana* is often nodulated in the field with the Sp^+ endophyte. Ecological conditions in addition to the role of the eventual host plant lead to selection of strains and therefore to their particular distribution. Nevertheless, it would be possible to introduce a new *Frankia* strain via nodulated alder after a careful study on its aptitude to compete with indigenous strains and its capacity to persist and survive despite biotic and abiotic factors. Such a strain must first be selected under greenhouse conditions with tests on the same soil type as that to be used for final transplanting.

Acknowledgements—We thank Dr Rodriguez-Barrueco C. (Salamanca, Spain) for critical reading of the manuscript. F. Kurdali received a fellowship from the Atomic Energy Commission of Syria.

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