

Cephalobus parvus as a carrier of *Rhizobium japonicum* in field experiment on soybean cultures

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SUMMARY

Two methods of soybean seeds inoculation by *Rhizobium japonicum* have been compared in a field experiment : *i*) traditional use of inoculated turf embedding the seeds; *ii*) use of *Cephalobus parvus* as bacterial carrier (3 000 and 300 000 nematodes per linear meter). There is no difference in yield between the inoculation methods in spite of a greater number of bacterial germs provided by nematode inoculation probably because there is a limit to the number of bacteria that can be used by one soybean seed. Any bacterium above this limit cannot be used by the seed. With turf inoculation all the nodules are localized at the root collar whereas with nematode inoculation they are regularly distributed on the rootlets. This is beneficial in case of soil surface drying which destroys nodules at the root collar.

RÉSUMÉ

Cephalobus parvus utilisé comme vecteur de *Rhizobium japonicum* dans une expérimentation en plein champ sur culture de soja

Deux méthodes d'inoculation de semences de soja par le *Rhizobium japonicum* ont été comparées dans un essai conduit en plein champ : *i*) inoculation classique par enrobage des semences; *ii*) inoculation au moyen de *Cephalobus parvus*, vecteur de bactéries (3 000 nématodes au mètre linéaire et 300 000 nématodes au mètre linéaire). En ce qui concerne la récolte, il n'y a pas de différence entre les deux modes d'inoculation, bien que le nombre de bactéries apportées soit beaucoup plus grand dans le cas de l'inoculation par nématodes. On peut expliquer ce phénomène par le fait que les besoins en bactéries pour une graine de soja sont limités à un seuil spécifique; au-delà de celui-ci, la graine ne retire aucun avantage. En ce qui concerne la répartition des nodules sur le système racinaire, lors d'inoculations classiques (enrobage) tous les nodules sont localisés au collet, tandis que lors d'inoculations par nématodes ils sont répartis sur toutes les radicelles. Cela est particulièrement utile en cas de dessèchement de la zone superficielle du sol, les nodules étant détruits s'ils sont limités au collet.

Because the soybean bacterium *Rhizobium japonicum* is not found in French soil it is necessary to inoculate the soybean seeds with it before sowing. The traditional inoculation method used by French farmers consists of embedding the seeds in a moist turf matrix that previously had been inoculated with *R. japonicum*.

Few studies have been published on the bacterial carrier ability of nematodes. Jatala, Jensen and Russel (1974) reported that the free-living nematode *Pristionchus lheritieri* (Maupas) is able to carry *R. japonicum*. We have indicated in a previous paper (Cayrol, Couderc & Evrard, 1977) that *Cephalobus parvus* (Thorne) and *Macrolaimus crucis* (Maupas) can be cultivated on monoxenic cultures of *R. japonicum* and *R. meliloti* and may act as carriers for these bacteria on seedling grown in the laboratory (on sand and agar medium).

The present study compares, under field conditions, the traditional inoculation method with inoculation using the free-living nematode, *C. parvus*, as a *Rhizobium* carrier.

Materials and methods

NEMATODE CARRIER

C. parvus was cultivated in Petri dishes on monoxenic cultures of *R. japonicum* on a specific agar medium (K_2HPO_4 : 0.5 g; $MgSO_4 \cdot 7H_2O$: 0.2 g; NaCl : 0.2 g; $CaCO_3$: 0.1 g; Mannitol : 10 g; Water yeast : 100 ml; Agar-agar : 15 g/1 000 cm³ dist. water; pH : 6.5).

Aseptic larvae of *C. parvus* were obtained by washing eggs of this nematode successively in mercuriothiolic acid 1/1000, then streptomycin sulfate 7/1000 during fifteen minutes. The disinfectants were then replaced by sterile water in which hatching occurred after three days. Twenty five newly hatched nematode larvae per Petri dish were inoculated on three days old *R. japonicum* cultures. The cultures were then incubated at 20°. Two months after inoculation the mean population was about 400 000 nematodes per Petri dish.

TRADITIONAL EMBEDDING INOCULUM

The turf inoculated with *R. japonicum* is prepared in air-proof aluminium bags by Liph laboratories (113, avenue Lacassagne, 69003 Lyon, France).

EXPERIMENT

The experiment was conducted in 1980 in an experimental field of the CETIOM (Centre Technique Interprofessionnel des Oléagineux Métropolitains) in the Rhône Valley at Puygiron.

Four treatments were compared :

- Inoculation by turf embedding (Lipha laboratories).
- Inoculation by *C. parvus* (300 000 nematodes by linear meter).
- Inoculation by *C. parvus* (3 000 nematodes by linear meter).
- Control not inoculated.

Each treatment was repeated three times in plots 10 × 2 m in dimension with six sowing furrows. Inoculation and sowing of soybean (cv. Hodgson) was done on May 25th.

For inoculation by turf embedding the seeds were mixed with inoculated moistened turf in a plastic vessel just before sowing. With this method, there was 1.10⁶ bacterial germs per seed.

For inoculation with carrier nematodes, a suspension of nematodes in water was poured in the sowing furrow before burying the seeds. In order to obtain a regular distribution of the nematodes in the soil, one liter of nematode suspension per linear meter, was applied with a watering-can. The nematode suspension was prepared by first washing the *Cephalobus* culture plates with about 5 ml of water in order to obtain a very highly concentrated suspension. After estimating the number of nematode present in the concentrated suspension, this suspension was diluted to 300 000 or 3 000 nematodes per liter. This method added 88.10⁸ and 88.10⁶ bacteria per seed when using respectively 300 000 and 3 000 nematodes per linear meter.

The experimental field was cultivated according to current agronomic practices (weeding, irrigation). 68 days after sowing, five plants were collected for each treatment. The plants were carefully up rooted in order to obtain a maximum of rootlets. The numbers of nodules on roots and collar were counted on each plant. At the end of the culture (October 27th) the yields were recorded in the different treatments.

Results and discussion

The number of the bacterial nodules on roots and collar, and the yields in the different treatments are indicated in Table 1. We observe that with bacterial inoculation by turf embedding, all the nodules are

situated on the collar, and they are significantly more numerous as the number of carrier nematodes increases. On roots there is no significant difference between the two amounts of nodules. There is no difference in yields between the three inoculation methods in spite of the differences in numbers of nodules.

According to Catroux (pers. comm.) the major factor for productivity in a soybean plant is not the total number of nodules but the total weight of nodules per plant. In the experiment, nodules with turf embedding inoculum were bigger than nodules with carrier nematodes. The total weight of nodules per plant may have been similar in all treatments. It would be interesting in a future experiment to weigh the nodules in

Table 1
Distribution of the nodules and yields
Répartition des nodules et récoltes.

Treatments	Mean number of bacterial nodules per soybean plant		Yields (kg/ha)
	Collar	Roots	
Control without <i>Rhizobium</i>	0	0	1 665 (a)
Turf embedding inoculum	7.0 (a)	0.2 (a')	2 405 (b)
3 000 nematodes per linear meter	18.8 (b)	23.6 (b')	2 440 (b)
300 000 nematodes per linear meter	31.0 (c)	36.8 (c')	2 355 (b)

Statistical analysis (t test) between the three inoculation methods :

- (a)/(b) t = 3,02* (a')/(b') t = 5.44***
- (a)/(c) t = 7,76** (a')/(c') t = 2.78*
- (b)/(c) t = 2,56* (b')/(c') t = 0.95-

- no significant difference
- * significant difference (P = 0.05)
- ** highly significant difference (P = 0.01)

Average yield for the three inoculation methods : 2 400
Standard deviation : 2.74

Test F : No significant difference between inoculations methods
Control significantly different from all three inoculation methods.

order to verify this hypothesis. It is surprising that the greater number of bacterial germs inoculated when using carrier nematode method (100 to 10 000 times more than with turf embedding method) do not result in a better yield.

According to Mousain (pers. comm.) this phenomenon could be explained because there is an upper limit to the bacterial requirements for one germinating seed and the seed, cannot use bacteria in excess to this limit. However, the limit changes during root development and a greater number of germs can be used by the plant when it is older. In a future experiment carrier nematodes will not be used at sowing but later, when the root system is more developed and consequently more receptive to an increased quantity of bacterial germs.

Conclusion

This field experiment shows that it is possible to inoculate soybean seeds with *R. japonicum* by means of *C. parvus* as a bacterial carrier. When this method was

compared to the traditional inoculation method, differences were observed in the distribution of the nodules on the root system. With turf embedding inoculation the nodules were localized at the root collar, whereas with nematode inoculation the nodules were localized both at the collar and on the roots. This can be beneficial in case of soil surface drying that can destroy nodules located at the collar. Other experiments are needed to define the exact number of carrier nematodes required and the best period of inoculation.

Bacterial carrier nematodes can be seen as a reliable mean to obtain regular inoculation of bacteria.

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