Influence of pathogenic nematodes on nodulation and seed yield of soybeans in Senegal

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SUMMARY

Previous research has suggested that nematode infestation might be responsible for decreased nodulation and N_2 fixation of soybeans, (*Glycine max* (L.) Merrill), and for decreased seed yields. Soybean yield increases obtained through fumigation to reduce nematode populations could result from indirect effects such as stimulation of mycorrhizae or elimination of pathogens other than nematodes. The objectives of this research were to : 1) evaluate the effects of fumigation on the soil nematode population and on nodulation, N_2 fixation and seed yield of field-grown soybeans ; 2) verify whether nematode infestation of soybean roots reduces nodulation and N_2 fixation.

Field plots were fumigated with a 1,2-dibromo-3-chloropropane (DBCP, 75 %) at the rate of 50 liters/ha. The fresh weight was measured when plants were 26, 40, 50, 70 and 85 days old. Seed yield and seed N were determined when the plants were 100 days old.

Fumigation decreased population densities of seven species of plant-parasitic nematodes and increased nodule number per plant, nodule weight per plant, and seed yield.

Pratylenchus sefaensis Fortuner, 1973, the most abundant plant parasite nematode found at the test site was added to autoclaved soil in the greenhouse to elucidate the effect of nematode infestation upon nodulation and N_2 fixation by soybeans. Infestation of soybeans by *P. sefaensis* reduced nodulation and N_2 fixation suggesting that this nematode may have been responsible for the reduced nodulation and N_2 fixation observed in the field studies.

The results indicated that plant-parasitic nematodes can reduce nodulation, N_2 fixation and seed yield of field-grown soybeans. Fumigation reduced these detrimental effects of plant-parasitic nematodes.

RÉSUMÉ

Influence des nématodes phytoparasites sur la nodulation et la production du soja au Sénégal

Des travaux antérieurs avaient suggéré que l'infestation par des nématodes pouvait être responsable d'une diminution de la nodulation, de la fixation d'azote et de la production de graines du soja. L'augmentation de production, obtenue à la suite d'une fumigation destinée à réduire les populations de nématodes, pourrait provenir d'effets indirects comme la stimulation des mycorhizes ou l'élimination d'agents pathogènes autres que les nématodes. Les objectifs du présent travail étaient : 1) d'évaluer les effets de la fumigation sur la population de nématodes du sol et sur la nodulation, la fixation d'azote et la production du soja au champ, 2) de vérifier si l'infestation des racines de soja réduit la nodulation et la fixation d'azote.

Les parcelles expérimentales ont été traitées au 1,2-dibromo-3-chloropropane (DBCP, 75 %), à raison de 50 litres/ha. Le poids frais des plantes a été déterminé aux âges de 26, 40, 50, 70 et 85 jours. La production de graines et leur contenu en azote ont été déterminés à 100 jours. La fumigation diminue les populations de sept espèces de nématodes phytoparasites et augmente le nombre de nodules par plante, le poids des nodules et la production de graines.

Pratylenchus sefaensis Fortuner, 1973, le parasite le plus abondant sur le site de l'essai au champ, a été inoculé dans des pots de sol autoclavé maintenus en serre pour déterminer les effets des nématodes sur la nodulation et la fixation d'azote du soja. On note que l'inoculation du soja par *P. sefaensis* réduit la nodulation et la fixation d'azote, ce qui suggère que ce nématode peut être tenu pour responsable de la nodulation et de la fixation d'azote réduites observées au champ.

Ces résultats montrent que les nématodes phytoparasites sont capables de diminuer la nodulation, la fixation d'azote et la production de graines de soja. La fumigation réduit ces effets néfastes.

As many as 50 species of plant parasitic nematodes representing about 20 genera have been identified from soybean (*Glycine max* (L.) Merrill) fields (Taylor, Anderson & Haglund, 1958). Within plant roots, nematodes cause extensive tissue alteration and destruction, which leads to above-ground visual symptoms. Nematode damage to roots reduces the uptake of water and nutrients resulting in less plant growth and low seed yields (Good, 1973). Nematodes may reduce or prevent normal nodulation and N_2

fixation (Epps & Chambers, 1962). The yellow-colored leaves of nematode-infested soybeans is usually attributed to N deficiency from inadequate nodulation and N_2 fixation, and this yellow coloring of the leaves can be alleviated by adding fertilizer to nematode-infested soybeans (Ross, 1959).

Soil fumigation with 1,2-dibromo-3-chloropropane (DBCP) can eliminate a large portion of the plant-parasitic nematode population and simultaneously increase nodulation and N_2 fixation (measured by the acetylene reduction method) and seed yield of soybeans growing in nematode-infested soils (Germani, 1979). A similar beneficial effect of DBCP was found in soils infested by the nematodes in other studies (Germani & Dhéry, 1973). These results suggested that nematode infestations might be responsible for decreased N_2 fixation of legumes and resulting losses in yield, especially in soils with low N content which had not received any N fertilizer. However, the yield increases obtained through DBCP fumigation could also result from direct effects of this nematicide on plant growth independent of nodulation or from such indirect effects as stimulation of endomycorrhizae as reported by Germani, Diem and Dommergues (1980), or elimination of other pathogens.

The influence of nematodes on the N_2-fixing potential of legumes is not always adverse ; in some situations nematode infestation has even been reported to stimulate nodulation and N_2 fixation (Baldwin, Barker & Nelson, 1975 ; Hussey & Barker, 1976 ; Baldwin, Barker & Nelson, 1979). Thus, it appeared necessary to 1) evaluate the effect of the soil nematode population on nodulation and seed yield of field-grown soybeans, and 2) to verify whether nematode infestation of soybean roots decreases nodulation and N_2 fixation.

Materials and Methods

Field studies

The experimental site field was located at Guerina, Casamance, South Senegal. The soil was a ferrallitic sandy soil which had never grown soybeans previously and was naturally infested with the nematodes as indicated in Table 1. Fertilizer (31 kg P/ha and 100 kg K/ha) was applied on the experimental area. The experimental design consisted of four randomized, complete blocks with two treatments : 1) non-fumigated, inoculated with *Rhizobium japonicum;* 2) fumigated, inoculated with *Rhizobium japonicum.*

Fumigated plots received 50 liter/ha of 1,2-dibromo-3-chloropropane (DBCP, 75%). Fumigation was carried out four weeks before seeding using a fumigant injector. The *Rhizobium japonicum* (strain USDA 138) inoculum prepared as a dry powder, according to Dommergues, Diem and Divies (1979), was applied to the seeding rows at a rate of 15 kg/ha. Soybean cultivar 44A73 obtained from the Institut de Recherche Agricole du Sénégal was sown on July 14, 1979, at a spacing of 10 cm within the rows 60 cm apart.

The number and fresh weight of nodules and the fresh weight of plant shoots were measured at 26, 40, 50, 70 and 85-days after planting. Seed yield, expressed in kg/ha, was determined at the end of the growth cycle when the plants were 100 days old. Seeds were ground and analyzed for N by the Kjeldahl technique (Burris & Wilson, 1957). Soil and roots were sampled when plants were 90 days old and assayed for plant-parasitic nematodes using Seinhorst's method (1950).

Greenhouse studies

Pratylenchus sefaensis was grown on maize *(Zea mays* L.*)* roots in a greenhouse and extracted in a Seinhorst mist chamber (Seinhorst, 1950). Mixed populations (adults and juveniles) obtained from this procedure were used to inoculate the soybeans. Soybean cv. 44A73 was grown in 60 pots (1.5 dm^3) containing the same sandy soil as the field experiment which had been autoclaved. One seed was placed in each pot (six replicates) in a randomized block design and inoculated with one ml of a broth containing 2×10^9 cells of *Rhizobium japonicum* USDA 138. The soil in half the pots was infested by adding 6,000 *P. sefaensis* at the crown of one-week-old seedlings while the other pots served as controls. Samples were taken 19, 28, 34, 46 and 56 days after the nematode inoculation. At each sampling period nodules were counted and weighed, and acetylene reduction activity was estimated using the procedure of Hardy *et al.* (1968) with some modifications (Germani, Diem & Dommergues, 1980), and expressed as micromoles C_2H_2 reduced per plant per h (ARAP). Plant shoots roots and nodules were dried at 60° and weighed. Nematodes were extracted from roots placed in a mist chamber (Seinhorst, 1950) and numbers were expressed on a fresh root weight basis.

Results

Field

Fumigation significantly decreased population densities of seven species of plant-parasitic nematodes detected (Tab. 1). Simultaneously, fumigation significantly increased seed yields and nodulation expressed as number (Fig. 1) or weight of nodules per plant (Tab. 2).

Table 1
Effect of soil fumigation on numbers of plant-parasitic nematodes occurring in field-grown soybeans

	Nematodes /dm³ soil		*Nematodes/100 g roots (fresh weight)*	
	Fumigated	*Non-Fumigated*	*Fumigated*	*Non-Fumigated*
Pratylenchus sefaensis	2,300 *	30,185	9,147*	148,410
Scutellonema sp.	746 *	4,310	148*	1,034
Helicotylenchus sp.	985 *	8,290	763*	6,410
Xiphinema sp.	0 *	160	0	0
Criconemella sp.	0 *	130	0	0
Hemicycliophora sp.	0 *	175	0	0
Meloidogyne sp.	0	0	0*	9,392

* Significantly less than non-fumigated control ($P < .05$).

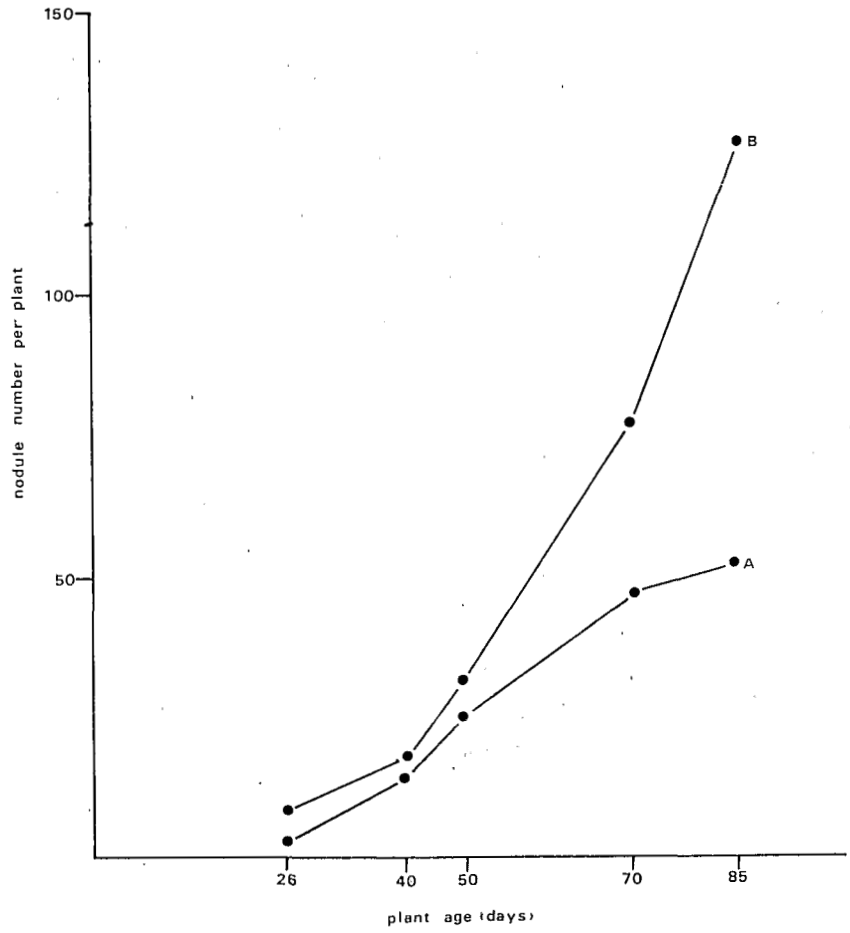

Fig. 1. Nodule number of field-grown soybean. Two treatments : A : non-fumigated, inoculated with *Rhizobium*, B : fumigated, inoculated.

Table 2

Effect of DBCP fumigation on number of nematodes *(Pratylenchus sefaensis)*, seed yield, seed N and nodule weight of 70-day old field-grown soybeans

	Non-fumigated	*Fumigated*
Nematodes/dm³ soil	30,200	2,300
Nematodes/100 g root*	148,400	9,150
Seed yield (kg/ha)	1,277	2,155
Seed N (kg/ha)	86	156
Nodules (g/plant)*	1.69	2.76

* Fresh weight basis.

Since DBCP fumigation dramatically reduced the population of *P. sefaensis*, it could be assumed that this plant-pathogenic nematodes was, at least to some extent, responsible for the decrease of nodulation and N_2-fixation in the field. However, other effects of soil fumigation can be hypothesized, as indicated in the introduction. The following experiment was set up in order to clarify the effect of nematode infestation nodulation and N_2 fixation by soybeans.

Greenhouse

Figure 2A shows that the infestation of soybean roots was successful since the number of *P. sefaensis* expressed on a root weight basis remained high throughout the entire experiment (more than 10^6 nematodes per 100 g of root). Infestation of soybeans by *P. sefaensis* reduced nodule number and nodule weight per plant in spite of the heavy *Rhizobium* inoculum (Fig. 2B and 2C). Nodule weight per plant was more affected than the number of nodules. The decrease in the number of nodules could be explained by a reduction in the number of infection sites on the root system. The more drastic reduction in nodule weight could possibly result from a secondary effect of nematode infestations, such as reduced photosynthesis or action of an inhibitor of nematode origin. Since nodule weight was reduced significantly the observed decrease in N_2 fixation as measured by ARAP (Fig. 2F) should be expected. Figures 2D and 2E show the harmful effect of nematode infestation on plant growth. The roots of infested plants were stunted (Fig. 3) and dark brown. This color was attributed to the necrosis resulting from nematode attacks.

Discussion

The field experiment reported here along with previous field experiments (Germani, 1979) shows that reduction of certain plant-parasite nematodes is a prerequisite for satisfactory soybean yields in West Africa under imperfect climatic conditions (the rainfall was only 892 mm in the experiment reported here). Controlling the population of *P. sefaensis* by fumigation increased the nodule weight. Since symbiotic N_2 fixation is primarily dependent upon the nodule weight of each plant, it can be assumed that, by impeding nodulation, nematode infestations reduce N_2 fixation. This conclusion is supported by the data of Ross (1959, 1969), who showed that chlorosis resulting from nematode infestations was alleviated by N fertilizer addition. Our field data indicate that fumigation enhanced nodulation and N_2 fixation.

The greenhouse experiment showed that non-infested plants were not only better nodulated but exhibited greater N_2 fixation (measured by the acetylene reducing activity) than plants infested with *P. sefaensis*. Thus, nematode infestation could be considered an important factor in the reduced nodulation and N_2 fixation observed in the field. It is interesting to note that the harmful effect of *P. sefaensis* is comparable to that of cyst or root-knot type nematodes as reported by Epps and Chambers (1962) in North America.

Seed N for the non-fumigated and fumigated treatments was 86 and 156 kg/ha, respectively. Assuming a harvest index for N of 0.8, the total N for the non-fumigated and fumigated treatments would be 108 and 195 kg/ha, respectively. Compared to the non-fumigated treatment, the fumigated treatment contained about 39% more nodule fresh weight, 41% more seed yield and 45% more N.

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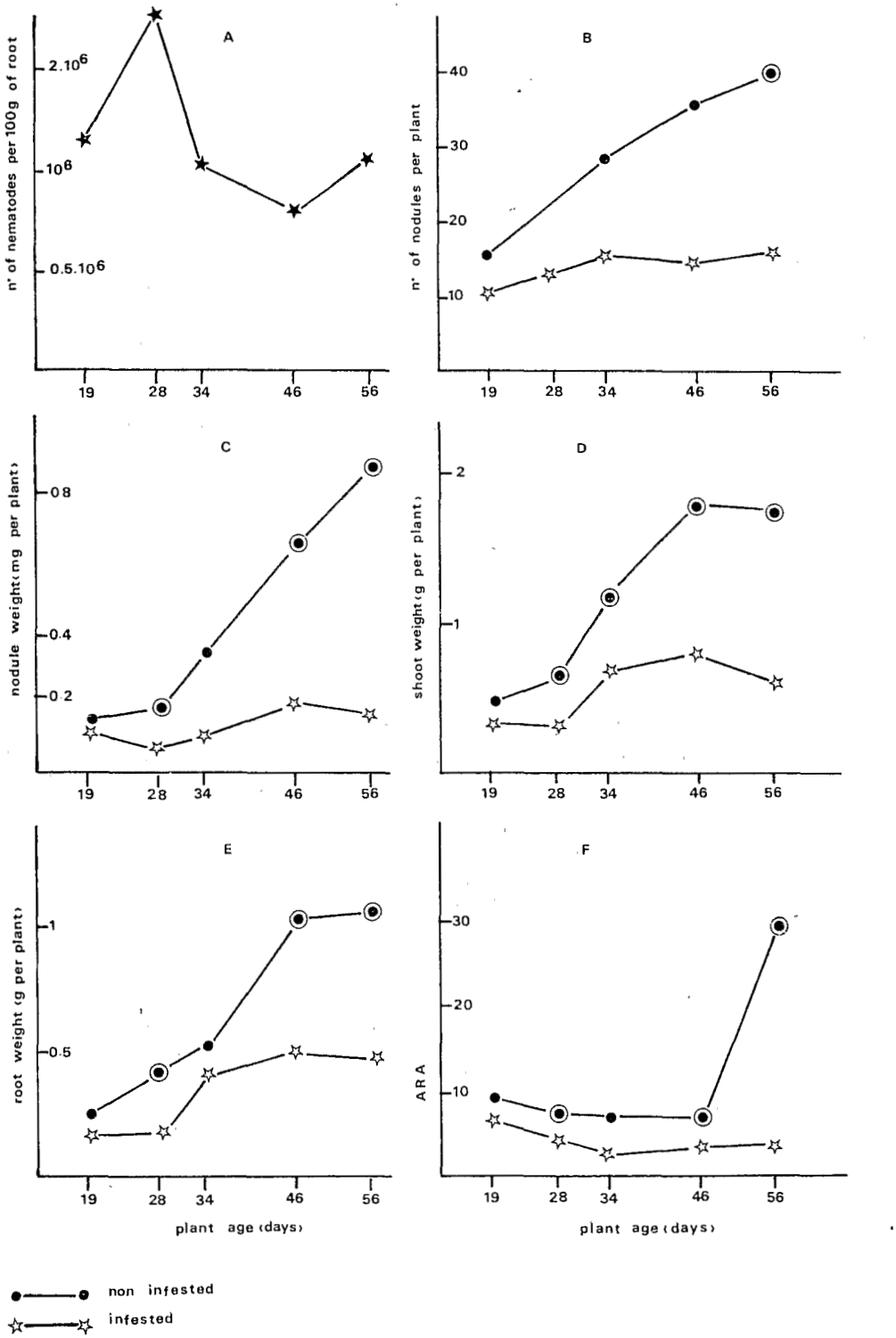

Fig. 2. Influence of infestation by *Pratylenchus sefaensis* on number of nematodes/100 g roots (A) ; nodulation (B, C) ; plant growth (D, E) ; ARAP, expressed as micromoles C_2H_2 reduced per plant per h (F). Significantly different data for same date are indicated by an extra circle.

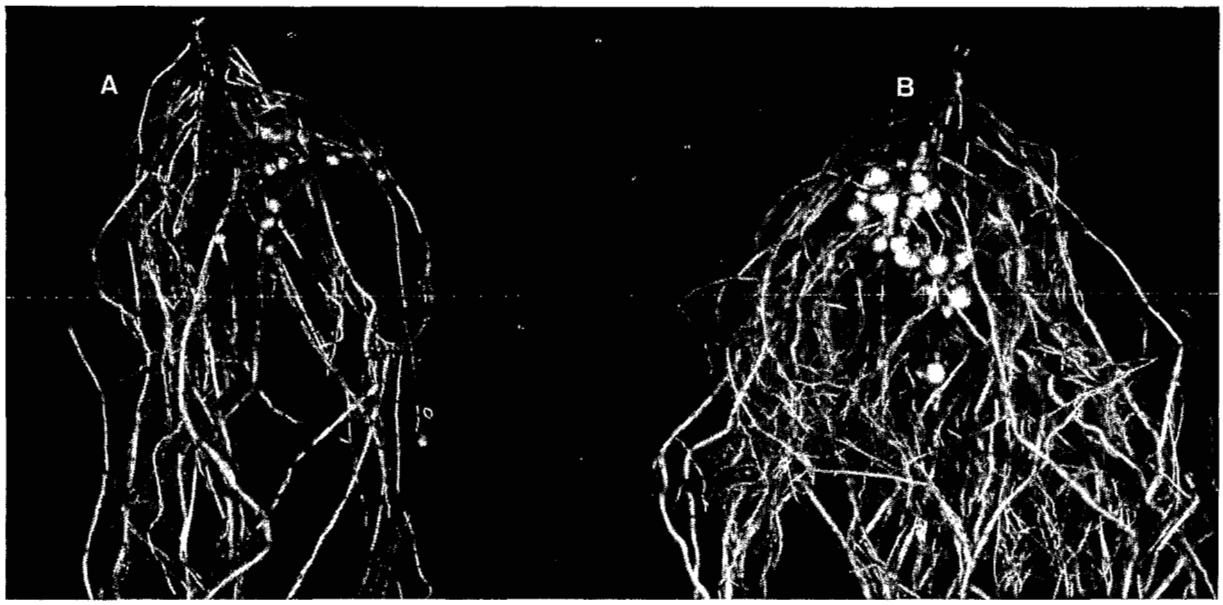

Fig. 3. Root system of 56-day old soybean infested (A) or non-infested (B) by *Pratylenchus sefaensis*.

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