NEMATICIDE APPLICATION AS A TOOL TO STUDY THE IMPACT OF NEMATODES ON PLANT PRODUCTIVITY

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A B S T R A C T

The disastrous effects of nematodes on the productivity of crop plants are often not recognized but they can be demonstrated under different farming systems, soil and climatic conditions by the use of nematicides. For example, two types of positive yield responses to DBCP (nematicide) have been observed with : (1) soybeans (infected by *Pratylenchus sefaensis* and *Aphasmastylenchus straturatus)* and groundnuts (infected by *A. straturatus* and *Scutellonema cavenessi*), and (2) groundnuts affected by clump disease caused by a virus transmitted by a fungus sensitive to the nematicide. DBCP treatment of a groundnuts chlorosis thought to be due to nematodes had, however, no effect on the disease or yields and it was subsequently established that the symptoms were due to K^{+}, Na^{+}, Cl^{-} toxicities resulting from the large amount of ash accumulating on the spots where plant residues were burnt at the time of land preparation.

Relatively few nematological studies have been carried out in vast areas of Africa, with its diversity of geographical and climatic régions. Many of the studies have been restricted to faunistic surveys. Investigations of fauna rarely suffice in themselves for the dectection of agricultural problems due to nematodes. This is especially true in the case of cultivated crops in Africa, which are often grown on recently cleared land where nematode populations are often very varied and contain new species.

High populations of polyphagic, pathogenic nematodes *(viz., Meloidogyne, Rotylenchulus* and *Pratylenchus* spp) and the presence of species specific to certain crops (*viz., Radopholus similis* on banana and *Tylenchulus semipenetrans* on citrus) are almost always associated with crop damage. This is not always the case with other species whose pathogenicity is unknown because of their recent discove-

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ry and a lack of information on their biology. Crop damage due to nematodes is often difficult, if not impossible, to discern through observation of the plants alone. Nematicide treatments are generally of great use in the detection of plant disease by nematodes.

There are two types of situations in which nematicidal applications are useful for the detection of disease problems in Africa : The first situation is where the nematodes found in a particular area are already known to be pathogenic in other areas of the world. Then nematicide treatments allow for the confirmation of pathogenicity and of the efficacy of the nematicide applied in the new situation. This is the case with banana infected by *Radopholus similis* in the Ivory Coast (Luc and Vilardebo, 1961) and of soybean in Senegal, infected by *Pratylenchus sefaensis* (Fortuner, 1973).

The second situation is where the nematodes infesting a given crop are not known or are only suspected of parasitism, and nematicide treatments restore or improve crop yield. The resulting benefits may be attributable to the control of nematodes. This is the case, for example, of a disease, " Upper-Volta chlorosis of legumes " (Germani, 1972) which was discovered almost at the same time as the possible causal agent *Aphasmatylenchus straturatus* (Germani, 1970). If nematicidal trials had not been carried out, it is likely that investigation of the nematode would have merely remained a taxonomic study because of its limited distribution and the relatively low level of its population in the soil.

Sometimes, however, yield increases resulting from nematicide application cannot be attributed directly to nematicidal activity.Most nematicides are rather general biocides and they can therefore affect some other pathogenic agents. If crop improvement does not occur with nematicidal treatment, however, it can be concluded that nematode pathogenicity is not a factor and attention can be directed to other causes. One case in point is where we were able to show that a specific chlorosis of peanut was due to soil physical factors and not to nematodes or other pathogens.

The following examples from our experiments and studies in West Africa will illustrate the use of nematicides for the detection or elimination of nematodes as causal agents of plant disease problems.

SOYBEAN INFECTED BY *Pratylenchus sefaensis*

A trial of nematicidal treatment with Nemagon (dibromo-chloropropane) was included in an experiment carried out at Sefa (Casamance) by ISRA* in the study of inoculation techniques of *Rhizobium* on soybean (cv. 44A73). In this experiment, six plots were treated with nematicide and six untreated served as controls. Each of the twelve plots received the same inoculation of *Rhizobium japonicum* G_2Sp embedded in a polyacrylamide gel (Dommergues *et al.*,1979). Three series of measurements were performed during the plant's life-cycle on the treated and control plots, *viz.* (1) the number and (2) dry weight of root nodules after 51, 67, and 88 days' plant growth; and (3) nematode count after 60 days of plant growth (Table 1). Data showed a considerable beneficial effect of the nematicide application on the nematode population and more particularly on *P. sefaensis*, which had been reduced to 27 per 100 g of roots as gainst an average of 200,000 for the control. The favourable effect of the nematicide was also apparent on the aerial parts of the plants. The root systems of plants in the control plots were less developed, had fewer nodules, and had a great deal of necrosis in comparison with the roots in the treated plots. The increase in yield of beans obtained on the treated plots was 106% that of the control plots (Table 1).

CHLOROSIS OF PEANUT AND SOYBEAN IN UPPER-VOLTA
INFECTED BY *Aphasmatylenchus straturatus*

At the begining of the 1960s, a chlorosis of peanuts had been noted in the village of Niangoloko in south-west Upper-Volta, but the cause was unknown. Observations carried out in 1968 had proved a consistent coincidence between the chlorosis and the presence of a new species of plant pathogenic nematode, *Aphasmatylenchus straturatus*, in the soil. It because evident later that not only peanuts but also other legumes growing in the same region shoved similar symptoms, *viz.* soybean, *Cajanus cajan*, *Tephrosia* sp., *Voandzeia subterranea*, *Vigna sinensis* and *Stylosanthes gracilis*. A survey on the extent and

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Table 1. Effect of soil fumigation with DBCP on number and weight of nodules and yield of field-grown soybean infected with *Pratylenchus sefaensis*.

		Number and dry weight (mg) of root nodules						
		51 Days		67 Days		88 Days		Yield
		Number	Dry weight	Number	Dry weight	Number	Dry weight	kg/beans ha
DBCP fumigated	(a)	27	181	31	407	33	276	2207
Unfumigated	(b)	14	174	21	333	21	291	1072
	a/b %	191	104	150	122	160	95	206

severity of the disease in peanut and *Cajanus cajan*, 1974, showed roughly 25% of the fields were afflicted with chlorosis and infested by *A. straturatus*.The affected areas comprised 1.7% of the total area observed.

The nematicide treatments performed on diseased peanuts always significantly decreased the numbers of nematodes, simultaneously suppressed the chlorosis (Plate 1, Fig. 3) and increased pod yields (Dhery *et al.*, 1975; Germani and Dhery, 1973). Foliar analysis showed that diseased plants were very deficient in nitrogen, the nitrogen level in healthy plants being 225% that of chlorotic plants. It was shown (unpublished data) that chlorosis of the legumes resulted from N deficiency and was correlated with reduced nodulation (Plate I, Fig. 1, 2) due to the parasitic action of A. *straturatus*. Another field experiment was made on soybean infected by *A. straturatus* in a field near the village of Koutoura (s.w. Upper-Volta). Four treatments were compared in this experiment (Fisher blocs design) (Table 2).

The calculation of the number and weight of the nodules and the measurement of ARAP (acetylene reduction per plant), carried out after 63 days' growth of the plants, and bean yield are shown in Table 2. The nematicide treatment had a greater influence on the yield than the *Rhizobium* inoculation. As noted previously in peanuts, infection of soybean by nematodes caused a significantly reduced nodulation, N_2 fixation and crop yield.

The application of nematicide on areas infected by *A. straturatus* has thus shown that a species belonging to a genus that was considered as a mere taxonomic curiosity could be a most serious pathogen. According to inoculation experiments carried out in the greenhouse, there is now evidence that *A. straturatus* is responsible for the chlorosis of legumes in Upper-Volta.

PEANUTS IN SENEGAL

A nematological survey in the traditional peanuts growing area in Senegal revealed that there was apparently no nematode specific only to peanuts and generally nematodes associated with them were the same as those infecting millet and sorghum, which are frequently grown in rotation with peanuts *Scutellonema cavenessi* Sher, 1963 peanut fields

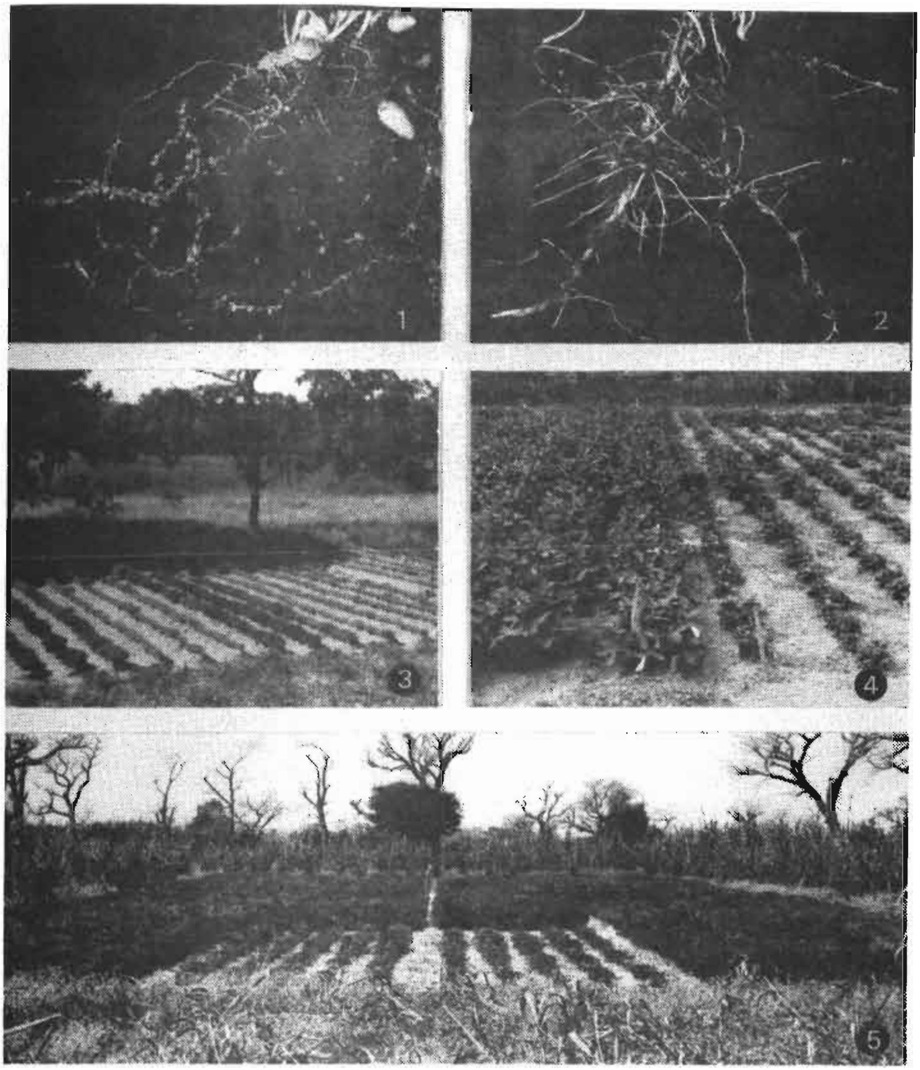

Fig.1: Upper-Volta chlorosis of peanuts: profuse nodulation in root system uninfected by nematodes. Fig.2: Upper-Volta chlorosis of peanuts poor nodulation in root system infected by *A.straturatus*. Fig.3: Upper-Volta chlorosis of peanuts experimental alternate plots fumigated with DBCP show growth improvement while unfumigated ones do not. Fig.4: Upper-Volta peanuts clump-disease: experimental plots fumigated with DBCP show growth improvement (left) while unfumigated ones do not.Fig.5: Growth improvement in penauts infested by *Scutellonema cavenessi* at Patar, Senegal, following fumigation with DBCP. The plants in the foreground (stunted) were not fumigated.

indicated it may be an important parasite.

Two experiments were carried out at Patar (Plate 1, Fig.5), Central Senegal, in order to: (1) estimate the impact of nematode infection on the growth and yield of peanut, (2) estimate the residual effect of nematicides on peanut and millet production and (3) compare responses of some peanut cultivars.

The first experiment was placed in a field where crop rotation was as follows: peanut (1974), peanut (1975), millet (1976), penaut (1977) and millet (1978). The experiment was set up according to a Fisher block design. More details about experimental procedures are reported in another paper (Germani and Gautreau, 1977). Yield data (Table 3) showed that DBCP fumigation significantly increased peanut and millet yield. Moreover it was interesting to note that this treatment enganced mycorrhizal infection of peanut variety 28-206 (Germani *et al.*, 1979).

In the second experiment, a nematicide application was made, before sowing, in 1978 on peanuts, (cv. 55-437) in a field contiguous to that of the first experiment (latin square design with four treatments). Tested chelicals were: Mocap 10 G at 100 kg/ha (10% a.i. as Ethoprofos) spread over the field surface; (2) Furadan 10 G at 6 g/m (10% a.i. as DBCP) applied by injection into the soil at 15 l/ha; and (4) Control (no soil treatment).

All the treatments were performed the same day as the sowing. None of the nematicides used were phytotoxic to groundnuts. In previous experiments with Nemagon, a higher rate of 25 l/ha was used. As the nematicide is phytotxic when used at this rate, it had to be applied one week before sowing. Since it is not possible to apply nematicides before the beginning of the rainy season in the Sahel, the treatments were made at the first rainfall and sowing was made at the second rainfall, thus avoiding any phytotoxic effect. Since it has been shown that a delay of 8 days in sowing causes yield losses up to 46% (Germani and Gautreau, 1977), the fact that a lower rate of Nemagon (15 l/ha) used at the time of sowing is not toxic seems to be of interest because it avoids the planting delay, and the cost of the treatments is much lower and a widespread application of these treatments can be considered.

Table 2. Effect of soil fumigation with DBCP and inoculation with *Rhizobium japonicum* on nodulation, N_2-fixation (ARAP) and yield of field-grown soybean infested with *A. straturatus* (Upper-Volta).

		Rhizobium inoculated				Uninoculated			
		Nodules		ARAP micromoles	Yield kg/beans/ha	Nodules		ARAP micromoles	Yield kg/beans/ha
		Number	Dry weight	per plant		Number	Dry weight	per plant	
Fumigated	(a)	19	678	5.59	777	9	410	3.07	872
Unfumigated	(b)	7	249	2.75	418	5	102	0.39	495
a/b	%	298	272	203	186	185	400	786	176

ARAP : Acetylene reducing activity per plant (micromoles/hour)

Nematodes were enumerated in the different treatments after 34 days of growth of the groundnuts, *i.e.* the time when the number of nematodes in the roots was increasing (see Fig.6). Table 4 showd data on yields (pods and straws). The effects of Nemagon and Furadan on nematode numbers are not similar, while their effects on groundnuts yield is quite similar. This is due to the fact that the first compound is a fumigant acting by contact and the latter has a systemic action.

In conclusion, although these results of nematicide treatments cannot be positively attributed to the mere killing of nematodes, this hypothesis cannot be rejected and it should be confirmed by further experiments. In any case, the application of nematicides appears to be economically feasible, the profitability being increased by the residual effect of Nemagon, which may last for two consecutive years in groundnuts and millet fields. The effect of nematicide treatments varied from year to year (Tables 3 and 4), probably along with rainfall which, in Senegal, is the major limiting factor in groundnuts production.

CHLOROSIS OF PEANUTS IN SENEGAL

During our survey on groundnuts in Senegal, a number of chlorotic areas were recorded. A nematicide treatment was performed in two areas, each area having four plots. Two of the plots were treated while the other two untreated plots were considered as controls. Two types of chlorosis were discovered.

Chlorosis attributed to a nematode (S. cavenessi)

The nematicide treatment was performed in the area where peanut (cv 55-437) regularly showed symptoms of chlororis. These areas were heavily infested by *S. cavenessi*. Plants from plots treated with Nemagon showed a homogeneous green appearance, in contrast to the chlorotic plants in the control plots (Germani and Gautreau, 1977). No pathogenic nematodes were observed in treated plots whereas high numbers of *S. cavenessi* were found in the control plots (30,000 ± nematodes per 100 g of peanuts roots). Plants from treated plots were 3.5 times higher in ARAP and had well-developed nodules. Yields from treated plots were 36% higher than those of controls. These

Table 3. Yields of groundnuts (cv 28-206) and millet (cv *Sanio*) observed in the year of fumigation with DBCP and one or two years later.

	Year of fumigation	Year of crop	Yield (kg pods per/ha)		
			Fumigated (a)	Unfumigated (b)	a/b, %
Groundnuts	1974	1974	3630	2545	143
	1974	1975	2680	1920	140
	1975	1975	2950	1015	291
Millet	1976	1976	1805	1523	119
Groundnuts	1976	1977	1469	641	229
	1977	1977	1893	793	239
Millet	1976	1978	1153	936	123
	1977	1978	1469	1272	115

Table 4. Effect of soil treatment with three nematicides on the number of *Scutellonema cavenessi* present in soil after treatment and on peanut yields (kg pods or straw per ha).

Treatment	No. of *S. cavenessi* per 100 g soil	No. of *S. cavenessi* per 100 g roots	Wt. of pods kg/ha	Wt. of straw kg/ha
Control	3248	27567	2103	2768
Mocap	3504	2145	2252 (7)	3156 (14)
Nemagon	14	26	2504 (19)	3589 (30)
Furadan	3288	1250	2753 (31)	3579 (30)

Numbers in brackets represent % yield increase

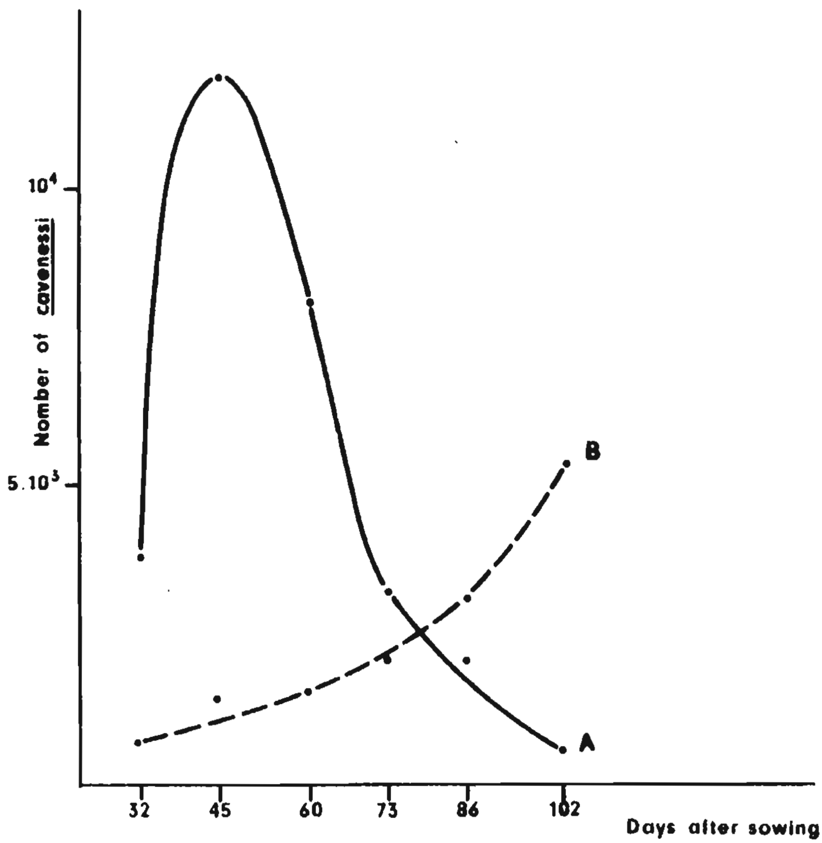

Fig. 6. Variations in the number of *Scutellonema cavenessi* per 100 g roots (A) or per litre of soil (B) during the growth of peanuts (*Arachis hypogea*) cv. 55-437 at Bambey Experimental Station, 1974.

results were similar to those obtained un Upper-Volta with *A. straturatus*.

Chlorosis attributed to a soil defect

This type of chlorosis was associated with the reduction of the root system and of nodulation. In this case, the nematicide treatment had no effect on the chlorosis. Nematological analysis of soil and roots showed that nematode populations were low even in control plots. It was noted that soil from the chlorotic areas contained a large number of charred plant fragments and ash. This suggested previous on-the-spot burning of bush or crop residue, a common pratice during the inter-season in Senegal, a factor which was most probably responsible for the peanuts chlorosis. This hypothesis was confirmed by experiments in the laboratory which showed that addition of ashes to the soil increased the pH up to 9.0 and simultaneously increased the Cl^-, K^+ and Na^+ content and induced chlorosis symptoms in peanuts (Germani, 1975).

CLUMP-DISEASE OF PEANUTS

Clump-disease of peanuts has been known since 1931 but its cause was discovered only in 1974, following nematicide treatment of diseased areas in Upper-Volta (Dhery *et al.*, 1975; Germani and Dhery, 1973) and in Senegal (Merny and Mauboussin, 1973). Although the soil treatment with DBCP suppressed the disease (Plate I, fig.4) and increased peanut yields (Dhery *et al.*,1975; Germani and Dhery, 1973), the responsibility of nematodes for this disease was not demonstrated. No nematode appeared to be clearly associated with the disease, and symptoms of clump disease did not resemble those generally caused by nematodes. Therefore investigations were initiated to determine whether the disease could be induced by a virus.

There is now evidence that clump-disease is virus-induced, as shown by graft transmission experiments and by electron microscopy (Germani *et al.*, 1974). Although positive results were obtained with nematicide treatments, they did not elucidate the nature of the pathogen (which was a tubular virus). They did, however establish that the vector was in the soil and also indicate that it was sensitive to DBCP. Attemps to study *Trichodorus* sp., the only nematode genus able to transport tubular virus in the rhizosphere, were not successful. It iq now suggested that the vector may be a fungus of the group *Plasmodiophorales* (Thouvenel, pers. comm.) whose spores are sensitive to Nemagon. The clump virus may also be transmitted by seeds (Thouvenel *et al.*,1978), which may explain its geographic dispersion.

Therefore experiments using nematicide treatments carried out in Senegal and in Upper-Volta on legumes and especially on peanut have led to some results of practical importance: *viz.*(1) the possibility of increasing peanut yields by applying a nematicide at the time of sowing, (2) the suppression of Upper-Volta chlorosis of peanuts (a disease affecting many legumes), (3) establishment of the nature of clump-disease and its control, (4) confirmation of the unfavourable effects of crop-residue burning on groundnut growth, and (5) better understanding of relationships between the host plant, *Rhizobium* , endomycorrhizae and pathogenic nematodes.

These studies have only been concerned with the traditional Sahel peanut growing areas in Senegal and in Upper-Volta. Further field experiments should be initiated in other Sahel zones to confirm the above date and possibly discover other diseases caused by nematodes and ways to effect their control.

DISCUSSION

Pereira : Is there any sharp evidence for the suppression of nematodes by maize ?

Germani : Maize is not a suitable host for *Meloidogyne* spp.

Bradley : How do nematodes affect nodulation ?

Germani : Possibly the nematode injects a toxin into the plant which inhibits nodulation or root hait formation. Preliminary experiments in which a nematode extract was injected into peanuts induced chlorosis.

Keya : Do you have any nematode-resistant variety of groundnuts ?

Germani : Not in the strict sense.

Ahn : The African marigold (*Tagetes* spp.) is known to suppress nematodes and is sometimes grown in a rotation for this purpose. How important could this be and what is the mechanism ?

Keya : But *T.minuta* is a very bad weed.

H.O. Mongi : *Tagetes minuta* need no longer be regarded only as a pernicious weed ! It is now the basis of a very lucrative perfume industry in southern Africa. Its cultivation in the areas affected by nematodes may, therefore, be exploited in that way.

Germani : The nematicidal effect of *Tagetes* spp. can be attributed to toxic compounds exuded by the root. Sesame is another interesting crop that seems to have similar properties.

Lal : Are some tree species that are recommended for planted fallows and for soil and water conservation, affected by nematodes ? If so, how do you control the nematodes ?

Germani : In Senegal, *Azadirachta indica* (Neem) is resistant to root-knot nematodes. Chemical control of three nematodes is known. *Tagetes* spp. can be grown as a cover crop.

Lal : How do tree species grown in association with food crops affect the incidence of nematode infection ?

Germani : In the presence of polyphagous nematodes, which are infrequent in Africa, tree roots may harbour pathogenic nematode species and thus act as reservoirs of inoculum. For example, papaya and baobab are good hosts for root-knot nematodes and *Rotylenchulus* spp.

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