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Influence of nematicide fumigation on mycorrhizal infection
and N₂ fixation in field-grown groundnut

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I. Introduction
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In the semi-arid conditions which prevail in psamment soils of West Africa, crop yields are limited not only by water but also by nematode attacks. These pathogens are well known to directly reduce crop yields by affecting the plant itself.

But they can also be harmful to it by decreasing the endomycorrhizal infection of the roots (Fox and Spasoff, 1972; Schenck and Kinloch 1974, Schenck et al. 1975) and, in the case of legumes, by reducing nodulation (Hussey and Barker, 1976; Yeates et al. 1977) and, consequently, N₂ fixation (Baldwin et al. 1975, Germani unpublished). Thus it is not surprising that fumigation of nematode-infested soils by some nematicides (1,2 dibromo-3- chloropropane and 1,3 dichloro-propene and related C₃ hydrocarbons) should result in a significant increase of endomycorrhizal infection of cotton roots (Bird et al. 1974).

The aim of the present work is to evaluate the influence of fumigation of a nematode-infested soil with 1,2-dibromo-3-chloropropane (DBCP) simultaneously upon (1) endomycorrhizal infection of roots, (2) N₂ fixation (3) crop yields of 3 different cultivars of field-grown groundnut in order to check whether nematicide fumigation can simultaneously protect the plant itself against nematode and improve its nitrogen and phosphorus nutrition by restoring the symbiosis with endomycorrhizae and Rhizobium.

II. Material and methods
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Groundnut cultivars 28-206 (growth-cycle : 120 days) 55-437 (growth cycle : 90 days) and GH. 119-20 (growth cycle : 120 days) obtained from the Institut de Recherche Agricole du Sénégal were seeded at a distance of 15cm in rows 60cm



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apart at the Patar site, 25km south of the "Centre de Recherche Agronomique de Bambey" (Agronomic Research Center at Bambey), central Senegal, on July 23, 1977. Fertilizer (6: 20: 10, N: P₂O₅: K₂O mixture) was applied on the experimental area at the rate of 150 kg/ha.

The main experimental design was made of 9 randomized blocks, with 2 treatments : fumigated and non-fumigated with nematicide and each plot was composed of 3 rows of groundnut, one for each cultivar. Treated plots were fumigated at a depth of 20cm using a fumigant injector, 22 days before sowing. The rate of application was 25 l ha⁻¹ of commercial nematicide (Nemagon) containing 75% 1,2 dibromo-3-chloropropane.

The soil was a typical psamment soil of central Senegal (Dior) characterised by a very low exchange capacity (Charreau and Nicou 1971) and heavily infested with Scutellonema cavenessi. The experimental site had been previously cultivated with pearl millet according to the traditional pearl-millet/peanut rotation system in use in the region (Charreau 1974).

The crop was harvested on October 20, 1977 (cv. 55-437) and November 10, 1977 (cv. 28-206 and GH. 119-20).

In order to check the remanents of the fumigation effect, an auxiliary field experiment with 2 treatments (fumigation performed the year before; no fumigation) was set up close to the first experimental site. Since a detailed report of this experiment will be given in another paper (Germani, unpublished), most results reported here are related to the main experiments.

During the groundnut growth cycle the rainfall was low (287mm for the whole season, June-October) but relatively well distributed. Plant material and soil were sampled twice (16th and 60th day) in order to estimate endomycorrhizal infection, N₂ (C₂H₂) fixation and nematode numbers. In order to reduce the number of measures, only 5 samples, rather than 9, were used for each treatment. But crop yield estimations were based on the plant harvest of each of the 9 replicates.

Estimation of endomycorrhizal infection

Five root systems per plot were examined.

Roots were stored in formalin-acetic acid-ethanol^{1/} and then treated and stained according to the method of Phillips and Hayman (1970). A microscopic examination was made under 250 x magnification on the total length of each 1cm root segment. For each root system, 12-15 segments were observed. The extent of mycorrhizal infection was expressed as percentage of microscope fields with endomycorrhizal infected roots.

Estimation of N₂ (C₂H₂) fixation

The basic procedure for the measurement of N₂ fixation was that of Hardy et al. (1968), with some modifications. Immediately after harvest, root systems were placed in 570ml serum bottles with a rubber stopper. Through the stopper, 50ml acetylene and 0.4ml propane (tracer gas) were injected.

Incubations were allowed to proceed for 30 min at ambient temperature (ca. 28°C). The incubation atmosphere was sampled using a 10ml Vacutainer tube. The ethylene assay was performed using a hydrogen flame ionization chromatograph (Varian aerograph 1200). The stainless steel column 150 x 0.3cm was packed with Spherosyl X OB 075 80-100 mesh + 10% Na₃PO₄. The injector, column and detector temperatures were respectively 105, 40 and 180°C. N₂ flow 30ml min⁻¹; H₂ flow 30ml min⁻¹; compressed air flow 300ml min⁻¹.

Acetylene reducing activity per plant (ARAP) was expressed as micromole C₂H₂ reduced per plant per h.

Crop yields

Aerial parts and pods were harvested separately in order to determine their weight after drying at 70-80°C.

Enumeration of nematodes

Nematodes were extracted from fresh soil samples by elutriation (Seinhorst, 1962) and from roots by mist chambers (Seinhorst, 1950). Results were expressed as numbers of Scutellonema cavenessi per dm³ soil or 100g root (fresh weight).

^{1/} Formalin (13ml), acetic acid (5ml) 50% ethanol (200ml)

RESULTS AND DISCUSSION

1. Effect of nematicide upon nematode populations

Fumigation with 1.2-dibromo-3 chloropropane significantly reduced nematode (Scutellonema cavenessi) populations. The effect of the treatment was dramatic when fumigation was performed in the same year as that of the crop (main experiment). Moreover this effect was shown to last at least for two years (auxiliary experiment).

2. Effect of nematicide upon endomycorrhization

Mycorrhizal infection, as evaluated at the first sampling (16th day) was significantly increased by fumigation for 2 cultivars (28-206 and GH 119-29).

Data presented in table 4 suggests intravarietal differences between the 3 cultivars since cv. 55-437 showed a tendency to resist endomycorrhizal infection when compared to other cultivars.

Since the groundnut (cv. 28-206 and GH 119-20) roots were heavily infected in the fumigated plots as soon as the 16th day, endomycorrhizal infection of some cultivars appeared to be a precocious process in the edaphic and climatic conditions occurring in Senegal, provided that no limiting factor - such as nematode attack - occurs. Late sampling (60th day) did not show any significant difference between treated and untreated plots. Thus, the nematicide treatment promoted only early endomycorrhizal infection.

In the case of cultivars 28-206 and 55-437, the effect of nematicide fumigation lasted for 2 years : endomycorrhizal infection, as determined on the 16th day of the growth cycle, did not significantly differ when fumigation was performed either just before, or one year before the groundnut crop (Table 4). Such a residual effect is probably related to the residual effect of fumigation on nematode populations already mentioned.

These results support the conclusion of the work of Bird et al. (1974) on cotton, that nematodes might eventually limit mycorrhizal infection, but the related mechanism is still unknown.

Microscopical observations showed that only very few vesicles and arbuscules were found in roots of groundnut in accordance with the report of Ross and Harper (1973).

3. Effect of nematicide upon N_2 (C_2H_2) fixation

Tables 1, 2 and 3 show that no significant effect on N_2 fixation could be detected at both sampling dates : 16th day and 60th day.

However, time course curves of Acetylene Reducing Activity per plant (ARAP), to be published elsewhere (Germani, personal communication), indicate that ARAP of cv. 55-437 and GH 119-20 in fumigated plots was markedly higher than that occurring in non-fumigated plots when plants were 40-50 days old, but that such a difference did not show up when plants were either 16 or 60 days old (Germani unpublished). Thus, nematicide fumigation appeared to improve ARAP of cultivars 55-437 and GH 119-20 but not that of cv. 28-206. It is surprising to note that whereas endomycorrhization of cv. 28-206 was not improved by fumigation, its ARAP was greatly enhanced by this treatment, at least during the period ranging from day 40 to 50 when ARAP is known to be the highest.

4. Effect of nematicide upon crop yields

The effect on crop yields expressed on a dry weight basis (Table 1, 2, 3) was dramatic, confirming previous experimental results already obtained by Germani (unpublished) in Upper Volta and Senegal.

Analyses of nitrogen and phosphorus of the plant material of the present experiment are under way. They are aimed at checking the results of field experiments performed in Upper Volta which indicated that nematicide fumigation increased crop phosphorus content, suggesting that restoration of endomycorrhizal infection might improve the crop phosphorus uptake.

In conclusion, by altering the physiology of groundnut, Scutellonema cavenessi presumably impedes the establishment and functioning of the double plant symbiosis (Mosse et al. 1976, Daft and El-Giahmi 1976) (1) with endomycorrhizae (2) with

Rhizobium, thus indirectly reducing plant phosphorus uptake on the one hand, and possibly its water uptake (Safir et al. 1971) and on the other hand, its N₂ fixing ability. Such consequences are presumably most harmful to the plant especially in phosphorus and nitrogen deficient soils which are largely found in semi-arid West Africa.

TABLE 1

Effect of soil fumigation with nematicide on nematode population, N₂ fixation, endomycorrhizal root infection and yields of field-grown groundnut cv. 28-206

	Nematicide (Nemagon)	
	No application	Application (25 l ha ⁻¹)
<u>Early sampling (16th day)</u>		
Number of nematodes dm ⁻³ soil	828	0 ***
Number of nematodes 10 ⁻² g root	1450	0 ***
ARA	4326	12007 NS
% mycorrhizal roots	22.8	59.6 **
<u>Late sampling (60th day)</u>		
Number of nematodes dm ⁻³ soil	3892	80 ***
Number of nematodes 10 ⁻² g root	15688	4 ***
ARA	9706	5498 NS
% mycorrhizal roots	63.6	40.7 NS
<u>Crop yield (harvest)</u>		
Pods (d.w.) kg ha ⁻¹	666	1592 ***
Aerial parts (d.w.) kg ha ⁻¹	1253	2587 ***
Seed N kg ha ⁻¹	24.46	58.55 ***

ARA : acetylene reducing activity expressed as nanomoles C₂H₂ reduced per plant per h

*** : Significantly different (P = .01)

** : Significantly different (P = .02)

NS : Not significantly different (P = .10)

TABLE 2

Effect of soil fumigation with nematicide on nematode population, N₂ fixation, endomycorrhizal root infection and yields of field-grown groundnut cv. 55-437

	Nematicide (Nemagon)	
	No application	Application (25 m ha ⁻¹)
<u>Early sampling (16th day)</u>		
Number of nematodes dm ⁻³ soil	848	0 ***
Number of nematodes 10 ⁻² root	688	0 ***
ARA	6228	9800 N S
% mycorrhizal roots	28.4	33.0 N S
<u>Late sampling (60th day)</u>		
Number of nematodes dm ⁻³ soil	2148	16 **
Number of nematodes 10 ⁻² g root	33362	0 ***
ARA	3941	7176 N S
% mycorrhizal roots	54.0	56.2 N S
<u>Crop yield</u>		
Pods (d.w.) kg ha ⁻¹	667	1606 ***
Aerial parts (d.w.)	1162	4400 ***
Seed N kg ha ⁻¹	28.92	64.21 ***

For explanation of abbreviations see Table 1

TABLE 3

Effect of soil fumigation with nematicide on nematode population, N₂ fixation, endomycorrhizal root infection and yields of field-grown groundnut cv. GH 119-20

	Nematicide (Nemagon)	
	No application	Application (25 l ha ⁻¹)
<u>Early sampling (16th day)</u>		
Number of nematodes dm ⁻³ soil	968	0 ***
Number of nematodes 10 ⁻² root	1760	0 ***
ARA	4908	12082 NS
% mycorrhizal roots	53.8	76.5 ***
<u>Late sampling (60th day)</u>		
Number of nematodes dm ⁻³ soil	2704	4 ***
Number of nematodes 10 ⁻² root	26058	0 ***
ARA	5981	6665 NS
% mycorrhizal roots	57.4	57.0 NS
<u>Crop yield</u>		
Pods (d.w.) kg ha ⁻¹	774	1748 ***
Aerial parts (d.w.) kg ha ⁻¹	1879	4079 ***
Seed N kg ha ⁻¹	29.84	68.70

For explanation of abbreviations see Table 1

TABLE 4

Residual effect of nematicide fumigation on mycorrhizal infection
of field-grown groundnut expressed as percentage of infected roots
determined on 16th day.

Groundnut cultivar	No fumigation (control)	Fumigation (20 l ha ⁻¹)	
		Just before groundnut crop	One year before groundnut crop
28 - 206	22.8 a	59.6 b	79.8 b
55 - 437	28.4 a	33.0 a	53.8 a
GH 119 - 20	53.8 ac	76.5 b	62.4 bc

Numbers in a row not having the same letter differ, P = .01, by Legay et al. (1966).
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