

Effects of Nematicides on *Pratylenchus penetrans*, Soil Nitrification, and Growth of Flue-Cured Tobacco

C. M. Tu, C. F. Marks, J. M. Elliot*

Pest Management Research Centre, Agriculture and Agri-Food Canada, 1391
Sandford Street, London, Ontario NW 4T3, Canada

Received: 14 June 1996/Accepted: 26 August 1996

Brown-root rot of tobacco, found in tobacco districts of Ontario, is caused by the root-lesion nematode *Pratylenchus penetrans* (Cobb) Filt and Stek. An estimated 70% of flue-cured tobacco (*Nicotiana tabacum* L.) acreage was found to have this symptom in 1970.

Fumigant nematicides which have been used for the control of nematodes in flue-cured tobacco have shown to inhibit the transformation of NH_4^+ to NO_3^- in soil. Marks et al. (1972) found lower levels of NO_3^- -N in fumigated than in untreated soil during June. Elliot et al. (1974) found that levels of NH_4^+ -N in the soil tended to be higher and NO_3^- -N lower with the fumigant-treated soils. In a laboratory study, Koike (1961) showed that the fumigants resulted in a 4- to 8-wk delay of nitrification in soil. This study reports the effects of certain fumigant and non-fumigant nematicide treatments on nitrification in soil, on flue-cured tobacco, and on population of *P. penetrans*.

MATERIALS AND METHODS

The experiment was conducted on Fox loamy sand with 5 treatments replicated four times in a randomized block design. Each 0.01038-ha plot consisted of four-rowed plots (104 m²), the outside two being guard rows. The fumigant nematicides used were Telone C (1,3-dichloropropene and related C₃hydrocarbons 85%, and chloropicrin 15%), Vorlex (1,3-dichloropropene and related C₃hydrocarbons 50%, and methyl isothiocyanate 20%), and the non-fumigant nematicides, oxamyl [S-methyl N', N'-dimethyl-N

*Deceased

Correspondence to: C. M. Tu

(methycarbamoyloxy)-1-thio-oxamimidate], 25% liquid, and triazophos [1-phenyl-3-(O,O-diethyl-thionophos-phoryl)-1,2,4-triazole], 40% emulsifiable concentrate. Materials, rates and methods of application of the nematicides are given in Table 1.

Table 1. Rate and methods of nematicide application.

Treatment	Rate/ha	Method
Control	0	Row
Telone C	90L	Row
Vorlex	28L	Row
Oxamyl	0.56kg a.i.*	Foliar spray
Triazophos	8.4kg a.i. (40%EC)	Broadcast spray

* a.i.: Active ingredient.

The nematicides were applied on May 7. The row treatments with Telone C and Vorlex were applied, sealed in, and aerated as described by Elliot and Marks (1972). Oxamyl at transplanting was added to the water in the planter barrel and applied at 6 litres per 24.38-m row. The foliar sprays were applied in 1 litre of water per row with a hand sprayer. Triazophos in 4 litres of water was sprayed over the entire plot and incorporated into the soil to a depth of 14 cm with a rotovator.

Flue-cured tobacco, c.v. Delhi 34, was transplanted to the plot on May 29, and fertilized with 25.2, 67.5, 171.2, 38.2 and 30.9 kg of N, P, K, Cl and Mg/ha, respectively. All the N was supplied by ammonium phosphate. To differentiate between native soil N and fertilizer N as the source of NH_4^+ and NO_3^- in the soil, and to eliminate the effect of N uptake by the plants, the first row of each plot was not fertilized, and a length of approximately 6 m of the first and second rows was not planted. The third and fourth rows were fertilized and planted in the normal way.

Samples for chemical analysis of tobacco were taken from each harvesting of each plot. The lumina (centre stalks) were dried and ground to pass a 2-mm sieve. A sample was composited from the five harvestings of each plot in proportion to the percentage of the yield occurring in

each harvesting. Moisture was determined by drying a ground sample at 110°C for 3.25 hr, and chemical constituents were calculated on an oven-dry basis. The method of Griffith (1957) was used to determine total alkaloids, the method of Harvey et al. (1969) for reducing sugars, and the AOAC method (1970) was used for Chlorine.

Numbers of P. penetrans in the soil were determined 1 day before nematicides were applied, then every week from May 24 to the end of June and every 2 wk through July and August. The population density of root lesion nematodes in each plot was determined by collecting 20 soil cores, 20 cm in depth, with a 2-cm diam. sampling tube. Each sample was mixed thoroughly and 50 g of soil was used for nematode extraction by the modified Baerman pan technique (Townshend, 1963). The numbers of nematodes in the roots were determined by collecting two tobacco roots from each plot 1-2 days after completion of harvesting. Each root was washed, the secondary and feeder roots removed, and a 25-g sample (fresh weight) was used for nematode extraction. The root samples were placed in a mist chamber at 22°C and nematodes that had emerged after 2 wk were counted. The root samples were then dried in a forced-air oven at 85°C for 1 wk and the population of nematodes was expressed on the basis of numbers per gram dry weight of root. Levels of $(\text{NO}_2^- + \text{NO}_3^-)\text{-N}$ were determined at the time of sampling, and after incubation for 2 wk in an incubator at 28°C by procedures reported by Tu (1980). A portion of the soil was incubated with 200 ppm $(\text{NH}_4)_2\text{SO}_4\text{-N}$ for 2 wk. The moisture was maintained at 60% of the moisture-holding capacity. All results are expressed on an oven-dry basis. The arithmetic means of the triplicate counts from all of the triplicate examinations were used to calculate the level of nitrification.

Total N in green tobacco leaves was determined at two dates in August on discs cut from the 10th leaf on 10 plants per plot. The tobacco from the third row of the plot was harvested and cured in the normal manner. Yield was calculated and samples analyzed for total N as described by Elliot et al. (1972). Crop index was calculated by multiplying the yield per 0.405 ha by the grade index.

The nematode count data were transformed to $\log (x + 100)$ before analysis of variance. The log means (y) were then transformed by the method of Quenouille (1950), $x' = \text{antilog} (y + 1.15 s^2) - 100$. All data were subjected to analysis of variance, and Duncan's new multiple range test (1955) was used to determine significant differences among means.

RESULTS AND DISCUSSION

Telone C at 90 L/ha, significantly decreased nitrification activity at 0 and 2 wk incubation both in native organic nitrogen and $(\text{NH}_4)_2\text{SO}_4$ -N fortified samples (Table 2). Fumigant, Vorlex was inhibitory for 2 wk in both native organic nitrogen and N-source enforced samples. Triazophos was also inhibitory after 2 wk with N-source added samples. Treatment of soil with chemicals for the control of insects, nematodes, and other pests will result in alterations in the activity and population of

Table 2. Effect of nematicides on soil nitrification [$\text{ppm} (\text{NO}_2^- + \text{NO}_3^-)\text{-N}$] in tobacco soil.

Treatment	Rate /ha	Addition of N-source			
		0	0	200 ppm	$(\text{NH}_4)_2\text{SO}_4\text{-N}$
		Period of incubation (wk)			
		0	2	0	2
Control	0	24.1 ab*	42.4 a	19.6 ab	132.2 a
Telone C	90L	14.9 b	35.5 b	12.2 b	27.2 b
Vorlex	28L	33.5 ab	31.1 b	27.2 ab	46.5 b
Oxamyl	0.56kg	41.1 a	40.9 a	33.4 a	160.9 a
Triazophos	8.4kg	42.3 a	39.9 ab	34.4 a	39.1 b

* Values within columns indicated by the same letter are not significantly different at 5% level determined by Duncan's multiple range test.

microorganisms related to soil fertility (Bollen, 1961; Martin, 1966; Tu and Bollen, 1968; Tu, 1970; 1972; Tu and Miles, 1976). In Ontario, certain chlorinated hydrocarbon fumigants which effectively control nematodes (Mountain, 1960; Tu et al., 1995) are applied by injection into the soil one month before transplanting

tobacco in the spring or in the previous fall. Many studies (Elliot et al., 1972; Jenkinson and Powlson, 1970; Tu, 1972; Walcott et al., 1967) have indicated that soil fumigants and certain nematicides retard or inhibit nitrification of ammonium nitrogen.

The population densities of the root-lesion nematodes in the soil and roots were determined at harvest. The numbers of P. penetrans in soil and root were lower than those in the untreated control with Telone C and triazophos treatments (Table 3). At the end of the growing season, nematode population densities in the roots were higher in plots with the control treatments. Triazophos tended to give better control of nematode than Telone C.

The agronomic and chemical analyses are shown in Tables 3 and 4. The treatments that provided better yield than the control were Telone C and triazophos. Vorlex did not provide good control of the root-lesion nematode in this experiment and yields were not higher than that of control. The new nematicide, triazophos (an organophosphorus compound) provided good control of nematodes throughout the season and resulted in a higher yield and crop index. Telone C fumigant produced tobacco with fairly high levels of chlorine which usually has poor burning qualities.

The level of total nitrogen, reducing sugars, and alkaloids in the growing leaves were not significantly affected by the treatments, although the Telone C and triazophos treatments tended to give higher levels on yield and crop index of tobacco. With the exception of Telone C, total chlorine was lower in the nematicide treatments than at a similar level as the control.

There were considerable numbers of nematodes in the roots of tobacco in the control plots in the summer-fallowed area, especially at the end of the season. Population density of nematode at the beginning (on May 24) was 3000 per kg of soil, whereas Olthof et al. (1973) suggested that a pre-plant density of nematodes in excess of 2000 per kg will reduce yield and crop value.

The nematicide, triazophos, gave fairly good nematode control, and increased the yield and crop index markedly

Table 3. Effect of nematicides on nematodes in soil and roots, yield and crop indexes.

Treatment	Rate /ha	No. of <u>P. penetrans</u> /kg soil	/g dry wt root	Yield (kg/ha)	Crop Index
Control	0	4250 a*	5100 a	2499 b	672.3 b
Telone C	90L	1850 bc	3250 b	2679 a	733.1 a
Vorlex	28L	3300 ab	6100 a	2528 b	692.6 b
Oxamyl	0.56kg	2150 abc	6000 a	2623 b	712.8 b
Triazophos	8.4kg	500 d	850 c	2825 a	777.6 a

* Mean values within columns followed by the same letter are not significantly different at $p=0.05$ determined by Duncan's multiple range test.

Table 4. Effect of nematicides on quality and chemical characteristics of flue-cured tobacco.

Treatment	Rate /ha	Reducing sugars (%)	Total alkaloids (%)	Total nitrogen (%)	Total chlorine (%)
Control	0	18.6 a*	2.82 a	2.09 a	0.74 b
Telone C	90L	21.8 a	2.68 a	1.99 a	1.03 a
Vorlex	28L	20.7 a	2.85 a	2.06 a	0.78 b
Oxamyl	0.56kg	18.1 a	2.98 a	2.18 a	0.78 b
Triazophos	8.4kg	19.3 a	2.70 a	2.06 a	0.67 b

* Mean values within columns followed by the same letter are not significantly different at $p=0.05$ determined by the Duncan's multiple range test.

over the control. Bromide containing fumigants, such as Terroicide 30 (30% chloropicrin, 36% ethylene dibromide and 34% inert) and Dowfume W-85 (85% ethylene dibromide) for treating tobacco soils should be used with great care and only when the requirements of good cultural practices are respected. This certainly accentuates the importance of management practice in controlling undesirable residues. In Ontario, although we have available

satisfactory materials for nematode control without using the chemicals with undesirable characteristics, the evaluation and screening of new chemicals for nematocidal purposes should be continued.

Acknowledgments. The authors gratefully acknowledge the technical assistance of M. E. Beck, D. Bologna, G. Hietkamp and L. G. Scanlan. Telone C was supplied by DowElanco Chemical Co. of Canada Ltd.; Vorlex by Nor-Am Agricultural Products Inc.; oxamyl by DuPont of Canada Ltd.; and triazophos by Hoechst AG.

REFERENCES

- Association of Official Analytical Chemists (1970) Official methods of analysis. 11th ed. AOAC, Washington DC, p. 55.
- Bollen WB (1961) Interactions between pesticides and soil microorganisms. *Annu Rev Microbiol* 15: 69-92.
- Duncan DB (1955) Multiple range and multiple F tests. *Biometrics* 11: 1-42.
- Elliot JM, Marks CF (1972) Control of nematodes in flue-cured tobacco. *Can Dept of Agric Pub* 1465, 10 pp.
- Elliot JM, Marks CF, Tu CM (1972) Effects of nematocides on Pratylenchus penetrans, soil microflora and flue-cured tobacco. *Can J Plant Sci* 52: 1-11.
- Elliot JM, Marks CF, Tu CM (1974) Effects of the nematocides DD and Mocap on soil nitrogen, soil microflora, populations of Pratylenchus penetrans, and flue-cured tobacco. *Can J Plant Sci* 54: 801-809.
- Griffith RB (1957) The rapid determination of total alkaloids by steam distillation. *Tobacco Sci* 1: 130-137.
- Harvey WR, Stahr HM, Smith WC (1969) Automated determination of reducing sugars and nicotine alkaloids on the same extract of tobacco leaf. *Tobacco Sci* 13: 13-15.
- Jenkinson DS, Powlson DS (1970) Residual effects of soil fumigation and mineralization. *Soil Biol Biochem* 2: 99-108.
- Koike H (1961) The effects of fumigants on nitrate production in soil. *Soil Sci Soc Am Proc* 25: 204-206.
- Marks CF, Elliot JM, Tu CM (1972) Effects of deep fumigation on Pratylenchus penetrans, flue-cured tobacco, and soil nitrate content. *Can J Plant Sci* 52: 425-430.

- Martin JP (1966) Influence of pesticides on soil microbes and soil properties. In: Pesticides and their effects on soil and water. ASA Special Publication, Soil Sci Soc Am Inc. Madison, Wisconsin, pp. 95-108.
- Mountain WB (1960) Present knowledge of nematodes in Ontario and their control by chemicals. Proc 7th Annu. Meet Agric Pestic Tech Soc. Guelph, Ontario, pp. 6-12.
- Olthof HA, Marks CF, Elliot JM (1973) Relationship between population densities of Pratylenchus penetrans and crop losses in flue-cured tobacco in Ontario. J Nematol 5: 158-162.
- Quenouille MH (1950) Introductory Statistics. Pergamon Press, London, England
- Townshend JL (1963) A modification and evaluation of the apparatus for the Oostenbrink direct cottonwool filter extraction method. Nematologica 9: 106-110.
- Tu CM (1972) Effect of four nematocides on activities of microorganisms in soil. Appl Microbiol 23: 398-401.
- Tu CM (1980) Influence of five pyrethroid insecticides on microbial populations and activities in soil. Microb Ecol 5: 321-327.
- Tu CM, Bollen WB (1968) Effect of paraquat on microbial activities in soil. Weed Res 8: 28-37.
- Tu CM, Miles JRW (1976) Interactions between insecticides and soil microbes. Residue Rev 64: 17-65.
- Tu CM, Marks CF, Johnson PW, Gayed SK, Elliot JM (1995) Effects of pesticides on soil enzymatic activities, Pratylenchus penetrans populations, black root rot, and growth of flue-cured tobacco. J Environ Sci Health B30 (2): 141-162.
- Walcott AR, Liao FH, Kirkwood JI (1967) Effects of fumigation temperature and level of nitrate on microbial numbers, CO₂ production, and N transformations in an organic soil. Soil Sci 103: 131-138.