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THE SUPPRESSION OF *MELOIDOGYNE INCOGNITA* ON TOMATO BY GRAPE POMACE SOIL AMENDMENTS

by

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Summary. Soil amendments with grape pomace at 5, 10, 20, 30, 40 and 50 g/kg soil dosages, alone or combined with 100, 200 and 400 mg N/kg soil of urea or 250, 500, 750 and 1000 mg N/kg soil of calcium cyanamide, were tested for their suppressive effect on *Meloidogyne incognita* on tomato in three different glasshouse experiments. Grape pomace significantly reduced reproduction and galling of *M. incognita* at all the tested dosages. The nematicidal effect was increased by the addition of 400 mg N/Kg soil of urea and reduced by combination with calcium cyanamide.

Addition of agro-industrial wastes to the soil can exert a remarkable suppressive action on phytoparasitic nematodes (D'Addabbo, 1995). The release of toxic compounds, preformed or derived from the degradation of the wastes in the soil, and/or the multiplication of nematode predators and/or parasites on the organic substrate are supposed to be the fundamental mechanisms of this nematicidal action (Stirling, 1991).

Grape pomace is largely available in all the areas of grapevine cultivation and its use as a soil amendment could also represent a possible solution to the problem of the disposal of this residue.

The combination of other agro-industrial residues, such as olive pomace, with nitrogen fertilizers has been shown to have a nematicidal effect on root-knot nematodes (Rodriguez-Kabana *et al.*, 1995; D'Addabbo *et al.*, 1997).

Calcium cyanamide is an amide fertilizer that has a suppressive effect on the soil microflora at high dosages (Cornforth, 1971), and therefore its addition to a soil amended with organic matter could provide evidence of the role of the soil microflora in the nematicidal action of organic amendments.

Based on the previous considerations, three different glasshouse experiments were undertaken to ascertain the possible nematicidal action of grape pomace, alone or in combination with urea or calcium cyanamide, on *Meloidogyne incognita* on tomato.

Materials and methods

In the first two experiments dry grape pomace, alone or combined with urea, was added at the rates specified in Tables I and II to a sandy soil respectively sterilized or naturally infested by 32 eggs and juveniles/cm^3 soil (*Pi*) of an Italian population of *Meloidogyne incognita* (Kofoid *et* White) Chitw. host race 1. The third experiment consisted of the addition of grape pomace, alone or mixed with calcium cyanamide to sterilized sandy soil at the rates specified in Table III.

Clay pots (12 cm diam, 500 cm^3 soil) were filled with the mixtures and arranged in a randomized block design with six replicates for each treatment, in a glasshouse at 25±2 °C.

Four weeks later one tomato (*Lycopersicon esculentum* Mill.) seedling cv. Rutgers was transplanted into each pot. In experiments 1 and 3 after one more week tomato plants were inoculated with 10,000 eggs and juveniles (20 eggs and juveniles/g soil) (*Pi*) of the same population of *M. incognita* used in experiment 2.

Plants were maintained in the glasshouse for two months, randomizing the position of the blocks and at the same time repositioning each plant within a block every week, to avoid a block position effect and at the same time the factor position of the plant within the block.

At the end of this period plants were uprooted and height, fresh and dry top and root weight recorded. Root gall index was estimated according to a 0 - 5 scale, where 0 = no galls, 1 = 1 - 2 galls, 2 = 3 - 10 galls, 3 = 11 - 30 galls, 4 = 31 - 100 galls and 5 > 100 galls (Taylor and Sasser, 1978). Nematode final population density (*Pf*) and reproduction rate ($r = Pf/Pi$) in each pot were calculated by processing tomato roots with a 1% aqueous solution of NaOC1 (Hussey and Barker, 1973) and 500 cm^3 soil with the modified Coolen's method (Coolen, 1979; Di Vito *et al.*, 1985) and then counting eggs and juveniles in water.

All data were statistically analyzed by the analysis of variance followed by Fisher's least significant difference.

Results and discussion

Grape pomace did not affect the growth of tomato plants at all the tested dosages when used alone, but resulted in a significant increase of growth parameters, compared to the unamended control, if combined at dosages ≥ 40 g/kg soil with urea (Tables I - III).

Reproduction of *M. incognita* on tomato roots was always significantly suppressed by the addition of grape pomace to the soil (Tables I - III). No statistical differences were found among the incorporation rates in experiments 1 and 3, whereas in the second trial dosages ≥ 30

TABLE I - *Growth of tomato and reproduction of* Meloidogyne incognita *in soil amended with grape pomace at different dosages.*

Amendment rate (g/kg soil)	Plant growth parameters: Plant height (cm)	Plant top weight: fresh (g)	Plant top weight: dry (g)	Root fresh weight (g)	Nematode reproduction parameters: Eggs/g roots (x 1000)	Reproduction rate (*Pf/Pi*)	Root gall index
0	44.7	24.0	4.3	13.6	111.5	106.0	4.3
5	56.3	22.8	3.9	12.2	71.6	74.8	3.7
10	49.8	20.5	3.6	12.5	70.1	72.9	3.8
20	46.8	22.4	3.6	15.5	59.4	71.6	4.3
30	54.2	23.9	4.3	18.5	52.8	72.2	4.3
40	58.2	25.3	4.2	18.4	46.1	71.7	4.2
50	64.0	32.9	5.5	19.3	44.3	64.6	4.2
L.S.D. 5%	14.7	11.0	2.1	6.3	24.5	17.9	0.6
L.S.D. 1%	19.6	14.7	2.7	8.4	32.7	23.8	0.8

TABLE II - *Growth of tomato and reproduction of* M. incognita *in soil amended with grape pomace and urea at different dosages.*

Treatment		Plant growth parameters				Nematode reproduction parameters		
			Top weight					
grape pomace (g/kg soil)	urea (mg N/kg soil)	Height (cm)	fresh (g)	dry (g)	Root fresh weight (g)	Eggs/g roots (x 1000)	Reproduction rate (*Pf/Pi*)	Root gall index
–	–	34.7	14.5	2.0	10.1	113.2	116.2	5.0
5	–	31.8	12.5	1.9	9.3	91.4	89.2	4.8
10	–	36.3	16.1	2.3	9.4	80.6	79.1	4.7
20	–	30.5	16.2	2.2	11.8	64.4	80.6	4.3
30	–	29.0	17.7	2.3	12.3	68.9	76.0	4.0
40	–	37.5	20.8	2.8	13.9	67.8	71.9	3.8
50	–	38.0	18.8	2.5	12.4	54.0	68.1	3.7
5	100	39.0	16.3	2.6	12.8	79.2	89.8	4.7
10	100	32.5	16.4	2.1	12.7	72.2	87.0	4.5
20	100	36.5	18.8	2.7	12.2	70.3	85.6	4.3
30	100	34.8	22.4	3.2	14.5	58.1	82.0	3.8
40	100	43.8	29.2	3.6	16.0	53.9	83.4	3.5
50	100	41.0	33.2	4.5	15.1	58.9	76.2	3.3
5	200	31.3	12.3	1.9	11.7	67.9	83.7	4.5
10	200	37.0	23.1	2.9	14.5	63.3	80.4	4.2
20	200	37.0	19.0	2.2	13.9	63.7	78.4	4.0
30	200	37.5	19.4	2.7	12.5	61.2	76.4	3.7
40	200	43.2	24.0	3.1	15.6	53.5	76.6	3.3
50	200	42.7	22.4	2.9	12.6	48.2	59.2	3.0
5	400	28.2	15.8	1.9	9.0	73.8	71.0	4.2
10	400	29.0	10.8	1.5	8.5	70.3	67.1	3.8
20	400	35.2	17.1	2.1	11.5	31.9	46.3	3.7
30	400	36.3	23.5	2.9	16.2	28.2	47.4	3.5
40	400	40.5	22.4	3.1	15.1	28.0	45.8	3.2
50	400	38.8	22.8	3.3	13.7	22.8	38.1	2.8
L.S.D. 5%		6.7	7.5	1.0	3.4	15.7	13.6	0.6
L.S.D. 1%		8.9	9.9	1.4	4.5	20.8	18.0	0.9

g/kg soil were significantly more suppressive than the lowest amendment rates but without differences among them. Combination with 400 mg N/kg soil urea reduced number of eggs and juveniles and galls of *M. incognita* on tomato roots compared with the same rates of pomace alone, whereas no statistical difference was found at the lower dosages of the fertilizer (Table II). Nematode reproduction rate increased significantly when dosages ≤ 20 g/kg soil of grape pomace were added with calcium cyanamide whereas no difference was found at the highest amendment dosage (Table III).

Based on the data from experiments 1 and 2, the analytical relationship between amendment dosage and *M. incognita* reproduction rate was derived. The best fit to experimental data, as shown by the high correlation index and level of significance, was given by the power equation $y = a + b x^c$ (1) (Fig. 1), in which y is the reproduction rate of *M. incognita* (*Pf/Pi*) and x the dosage of grape pomace. From eq. (1) the reduction of a *M. incognita* population caused by amending the infested soil with a known dosage of grape pomace could be estimated. However, the equation should also fit data from experiments with other crops.

In conclusion, grape pomace demonstrated a remarkable suppression on *M. incognita* and no toxic effect on tomato, and these findings were confirmed in all three experiments. Moreover, the relatively low dosages needed and the large availability at low or zero cost make the use of this residue easy and immediate. Further, the nematicidal action can be enhanced by the use of organic nitrogen fertilizers at practically acceptable dosages.

In a previous experiment (Pelagatti *et al.*, 1993) addition of exhausted grape pomace was found to cause a significant increase of total soil microflora and in particular of some genera of nematode parasitic fungi (*Paecilomyces* spp., *Cla-*

TABLE III - *Growth of tomato and reproduction of* M. incognita *in soil amended with grape pomace and calcium cyanamide at different dosages.*

Treatment		Plant growth parameters				Nematode reproduction parameters		
			Top weight					
Grape pomace (g/kg soil)	Calcium cyanamide (mg N/kg soil)	Height (cm)	fresh (g)	dry (g)	Root fresh weight (g)	Eggs/g roots (x 1000)	Reproduction rate (*Pf/Pi*)	Root gall index
–	–	57.6	26.4	2.2	5.6	29.5	11.6	5.0
5	–	64.4	31.3	2.6	5.7	12.8	4.8	4.2
10	–	57.4	29.2	2.6	4.7	15.0	5.1	4.3
20	–	55.4	25.4	2.4	4.8	6.9	2.0	2.8
40	–	70.3	33.9	2.9	4.5	7.7	2.3	3.8
5	250	61.0	40.7	4.2	8.5	29.1	17.6	5.0
5	500	66.2	50.7	4.5	8.2	23.6	14.5	5.0
5	750	64.8	38.1	2.8	4.8	26.4	10.2	4.5
5	1000	60.0	50.8	4.4	5.6	25.8	11.4	4.8
10	250	60.9	48.0	4.6	6.8	20.3	9.8	4.8
10	500	61.8	58.0	5.8	9.0	13.6	9.6	5.0
10	750	57.6	49.6	4.9	7.0	22.4	11.7	4.8
10	1000	62.8	54.2	4.8	5.3	17.0	6.5	5.0
20	250	67.7	57.3	5.3	8.3	7.0	4.8	4.0
20	500	76.1	56.5	4.9	6.4	9.1	3.9	4.0
20	750	63.3	51.8	4.4	6.5	15.6	7.3	5.0
20	1000	59.2	53.3	4.6	6.3	16.9	7.9	5.0
40	250	66.0	50.7	4.8	6.9	14.2	2.8	4.2
40	500	68.2	54.6	5.0	6.8	7.5	3.4	4.0
40	750	69.5	69.2	6.3	8.6	4.7	3.1	4.2
40	1000	66.2	68.7	6.0	8.3	5.4	3.8	4.0
L.S.D. 5%		12.8	12.1	1.2	2.1	8.2	4.3	0.6
L.S.D. 1%		16.9	16.0	1.6	2.7	10.9	5.7	0.8

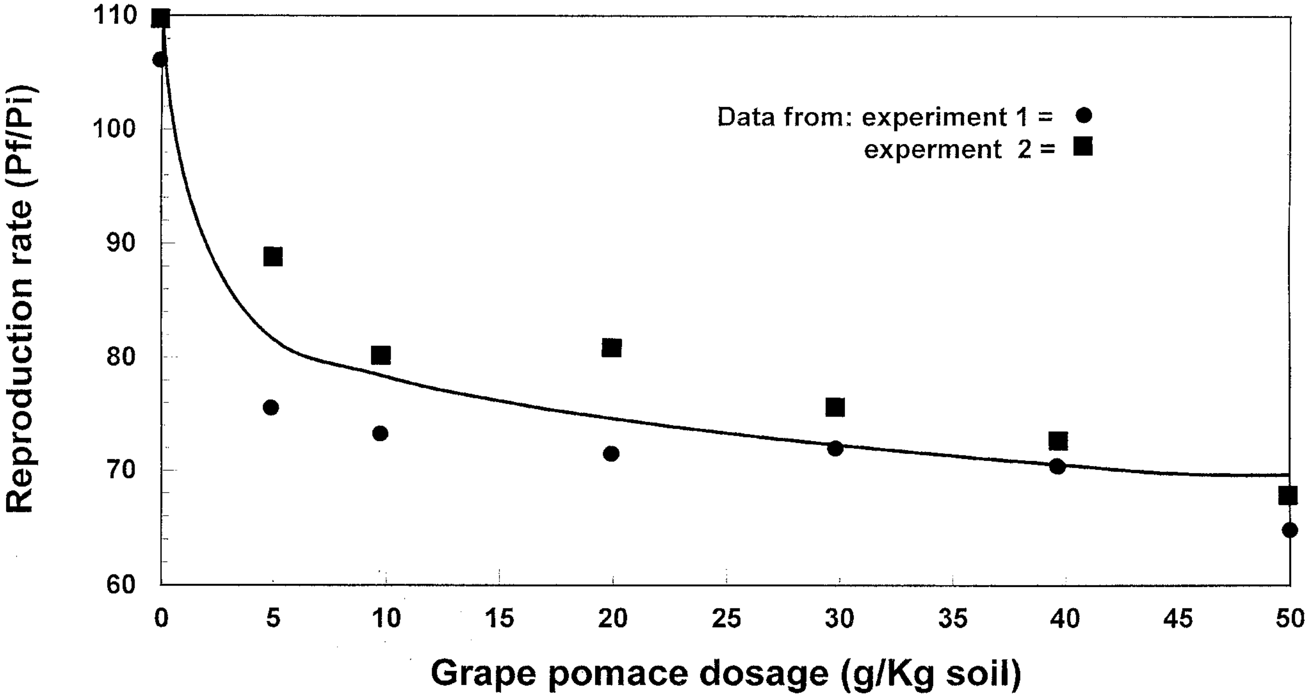

Fig. 1 - Relationship between dosage of grape pomace and reproduction rate of *Meloidogyne incognita* on tomato: $y=111.4-23.2x^{0.15}$; $r=-0.95$ ($P=0.05$).

dosporium spp.). The enhancing effect of urea and the reducing effect of calcium cyanamide on nematode suppression by grape pomace suggests that the nematicidal action is prevalently due to the development of microrganism parasites of nematodes on the organic substrate: urea provided the nitrogen necessary for the multiplication of these microrganisms, whereas the well known microbicidal activity of calcium cyanamide reduced their development on grape pomace.

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