Available online at www.sciencedirect.com
ScienceDirect
Crop Protection 25 (2006) 1244–1252
Crop Protection
www.elsevier.com/locate/cropro

Application of an emulsifiable mixture of 1,3-dichloropropene and chloropicrin against root knot nematodes and soilborne fungi for greenhouse tomatoes in Italy

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Received 23 August 2005; received in revised form 19 January 2006; accepted 27 March 2006

Abstract

A mixture of 1,3-dicloropropene 60.5% w/w and chloropicrin 33.3% w/w (Telone C35 EC) may be registered in Italy for soil drip fumigation. Five experiments on greenhouse tomatoes in Northern, Central and Southern Italy compared the effectiveness of this mixture in comparison with methyl bromide to find the optimum application rate in soils infested by *Fusarium oxysporum* f.sp. *lycopersici*, *F. oxysporum* f.sp. *radicis lycopersici*, *Sclerotium rolfsii*, *Meloidogyne javanica* and *M. incognita.* Its efficacy against *F. oxysporum* f.sp. *radicis lycopersici* and *M. incognita* was confirmed when applied to soils at 100, 200, 300 and 400 l ha^{-1} (132.4, 268.4, 402.6 and 536.8 kg ha^{-1}) under gas-tight films with 15–45 mm of application water (900–1200 mg Telone C35 EC l^{-1}). In sandy soils, with slight *F. radicis lycopersici* infections and with heavy nematode (*M. incognita*) attacks, the mixture, drip applied at 900 mg l^{-1} during late summer (fumigation: late summer; transplant: late-summer/autumn; last harvest: early spring), performed well up to 132.4 kg ha^{-1} (100 l ha^{-1}). In sandy loam soils with slight *F. radicis lycopersici* infections and severe infections of *F. lycopersici* and galling nematodes (*M. javanica*), 268.4 kg ha^{-1} (200 l ha^{-1}) of the mixture applied at 900 mg l^{-1} as a drip provided yields similar to those of methyl bromide treated plots both in spring and summer cycles. In sandy loam soils, the diseases (*F. lycopersici*, *F. radicis lycopersici*) were controlled at rates $\geqslant$268.4 kg ha^{-1} (containing 90 kg ha^{-1} of chloropicrin), but the mixture was ineffective against *Sclerotium rolfsii* occasionally observed in sandy loam soils. In both sandy and sandy loam soils, no significant relationships were found between the rates of mixture applied (132.4, 268.4, 402.6 and 536.8 kg ha^{-1}) and the degree of nematode infestation.

Keywords: *Fusarium oxysporum* f.sp. *lycopersici*; *Fusarium oxysporum* f.sp. *radicis-lycopersici*; *Meloidogyne* spp., *Sclerotium rolfsii*; Soil fumigation; Tomato

1. Introduction

Methyl bromide (MB) was phased out from January 1st 2005 in industrialized countries but is still available in developing countries as part of the Montreal Protocol (Gullino et al., 2003, 2005). For the year 2006, the Technology Economic Assessment Panel (TEAP) agreed that Italy could use 855 metric tons of MB distributed for the following crops: tomato (58%), strawberry (9%), cut flowers (9%), eggplant (5%), pepper (9%), melon (4%) and strawberry runners (7%), representing 12% of baseline consumption of MB in 1991 (6974 metric tons) with a further decline in the future. To evaluate alternative fumigants during the phase-out process of MB, several experiments have been performed in Italy as well as in other countries (Gullino et al., 2003). Chloropicrin and 1,3-dicloropropene (1,3-D) are among the most promising chemicals for controlling soilborne diseases and nematodes (Duniway, 2002; Martin, 2003). Chloropicrin

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doi:10.1016/j.cropro.2006.03.017

(trichloronitromethane) (CP), currently used as a warning agent in combination with odorless MB (Awuah and Lorbeer, 1991), has been registered in Italy since 2002 (Tripicrin™, emulsifiable formulation, 94% w/w active ingredient, 1607 g a.i./l formulation) (Ajwa et al., 2003; Gullino et al., 2003). CP is used at present as a soil fumigant in open fields and in greenhouses before planting crops such as strawberry, tomato, eggplant, pepper, melon, watermelon and zucchini (Gullino et al., 2003). CP can be applied by shank injection or, when emulsified formulations are used, through drip irrigation systems: this treatment is commonly named "drip fumigation" (Ajwa et al., 2002). Drip fumigation is a practical, economical and safe application strategy in greenhouses, where shank injection is not generally appropriate (Ajwa et al., 2002; Desaeger et al., 2004; Gan et al., 1998, 2000; Guo et al., 2005; Schneider et al., 1995; Wang and Yates, 1999). Drip fumigation avoids exposing workers in the greenhouse during fumigation and allows simultaneous or subsequent application of fumigants and/or water emulsifiable non-fumigant pesticides (Desaeger et al., 2004). In accordance with Italian law "Regio Decreto no. 147, January 9th 1927," CP is considered a "toxic gas" and it must be applied only by licensed applicators, similar to MB, and under virtually impermeable plastic films (VIFs) (Gullino et al., 2003).

The fumigant 1,3-D is an effective nematicide with fungicidal properties used worldwide (Duniway, 2002; Martin, 2003) and commonly applied alone as a fumigant for nematode control or in combination with CP to control soilborne diseases and weeds (Gilreath et al., 1994; Hutchinson et al., 2003). In open field. 1,3-D is applied as a broadcast shank-injected treatment followed by soil sealing (mechanical smearing or plastic tarpaulin). In Italy, an emulsifiable concentrate of 1,3-D (Telone 94 EC, 94% w/w active ingredient, 1161.8 g a.i. l^{-1}) was registered in November, 2001 and applied in greenhouses using drip irrigation (Gullino et al., 2003).

Mixtures of CP and 1,3-D are available commercially worldwide (Duniway, 2002; Martin, 2003), but two of the formulations are not yet registered in Italy. These contain 1,3-D with 17% CP (Telone C17) or 35% CP (Telone C35).

This paper describes experiments carried out in Italy under different environmental conditions in experimental and commercial farms. The experimental activities were carried out in fields infested with soilborne disease and root knot nematodes and were aimed at testing the effectiveness of an emulsified mixture of CP and 1,3-D (Telone C35 EC), expected to be registered for shank and drip applications in Italy. Trials were designed to find the optimum rate by drip and, because tomato protected crops constituted the most important sector for MB fumigation in Italy, the experimental activities were aimed at testing the effectiveness of the emulsified fumigation on protected tomatoes.

2. Materials and methods

2.1. Study sites

Four different sites hosted the experimental activities: two trials were carried out under glasshouse at Albenga (SV) in Northern Italy, one trial was conducted under plastic tunnel at Caivano (NA) in Central Italy and two trials were carried out under plastic greenhouse at Scoglitti (RG) and Acate (RG) in Southern Italy. Details relevant to the experiments are given in Tables 1 and 2.

2.2. Soil infestation with pathogens

Each experimental site had a history of at least one tomato crop during the three seasons prior to the beginning of this study. To achieve a uniform and high disease incidence, before fumigation at the Albenga site in 2002, *Fusarium oxysporum* f.sp. *lycopersici* Schlecht. and *Fusarium oxysporum* Schlecht f.sp. *radicis lycopersici* Jarvis et Shoemaker were incorporated into the soil. Two strains of each pathogen, freshly isolated from infected tomato plants, were grown on autoclaved wheat seeds. Twenty g m^{-2} of infested seeds for each pathogen were incorporated into the soil by rototilling to a depth of 10–15 cm 18 days prior to fumigation, keeping the soil moist by means of periodic sprinkler irrigation (5–10 mm). A similar procedure was successfully adopted during previous

Table 1
Relevant details and soil characteristics of the experimental sites

	Albenga (SV) Northern Italy		Scoglitti (RG) Southern Italy	Acate (RG) Southern Italy	Caivano (NA) Central Italy
Year	2002	2003	2003	2003	2003
Farm	Experimental[a]		Commercial	Commercial	Commercial
Total area devoted to the trial (m^2)	1100	1100	1100	2400	1200
Soil texture	Sand 75.0%		Sand 97.5%	Sand 90.9%	Sand 57.0%
	Silt 20.0%		Silt 0.8%	Silt 5.5%	Silt 29.0%
	Clay 5.0%		Clay 1.7%	Clay 3.6%	Clay 14.0%
pH	8.1		7.8	7.7	8.0
Organic matter (%)	2.5		1.0	1.8	1.9
Cation exchange capacity meq 100 g $soil^{-1}$	8.5		12.4	16.6	25.2
Soil classification	Sandy loam		Sand	Sand	Sandy loam

[a]Centro Regionale di Sperimentazione ed Assistenza Agricola (C.E.R.S.A.A.).

Table 2
Relevant trial dates

Trial	Site	Artificial inoculation	Fumigation with Telone C35 EC	Fumigation with MB	Tarp removal	Tomato transplant	End of the trial
1	Albenga 2002	28/02/02	17/04/02	16/04/02	06/05/02	13/05/02	31/07/02
2	Albenga 2003	–	03/07/03	08/07/03	30/07/03	04/08/03	11/12/03
3	Scoglitti 2003	–	03/09/02	03/09/02	23/09/02	25/09/02	02/03/03
4	Acate 2003	–	07/08/02	12/08/02	28/08/02	03/10/02	14/03/03
5	Caivano 2003	–	11/02/03	11/02/03	21/02/03	27/03/03	31/07/03

Table 3
Percentage of tomato plants infected by *F. lycopersici*, *F. radicis-lycopersici*, *M. javanica*, *F. lycopersici* and nematodes, *F. radicis-lycopersici* and nematodes in soil either untreated, or treated with 1,3-D/CP (C35) or MB (Trial 1, 2002—79 DAT)[a]

Treatment—rate $g\,m^{-2}$—amount of irrigation water	Only FOL[b]	Only FORL[c]	Only nematodes	FOL + nematodes	FOL and FOL + nematodes	Total
C35—13.42—15	2.3 a[d]	2.3 a	0.0 a	0.0 a	2.3 a	4.7 a
C35—26.84—30	1.5 a	3.9 a	0.0 a	0.0 a	1.5 a	5.5 a
C35—40.26—35	1.5 a	2.0 a	0.0 a	0.0 a	1.5 a	3.5 a
C35—53.68—45	0.8 a	2.3 a	0.0 a	0.0 a	0.8 a	3.1 a
MB—40	3.8 a	2.3 a	0.0 a	0.0 a	3.8 a	6.1 a
Control	3.6 a	1.5 a	17.8 b	9.1 b	12.7 b	32.0 b

[a] DAT = days after transplant.
[b] FOL = *F. oxysporum* f.sp. *lycopersici*.
[c] FORL = *F. oxysporum* f.sp. *radicis lycopersici*.
[d] Numbers in the same column followed by the same letter are not significantly different according to Tukey HSD test ($P = 0.05$).

experimental activities carried out at Albenga site under similar conditions achieving a satisfactory and uniform disease incidence on tomato (Gullino et al., 2002).

No artificial infestation was used to increase the population density of root knot nematodes. At the Albenga site (Northern Italy), in Caivano (Central Italy) and Vittoria (Southern Italy), soil samples were taken to asses the uniformity of infestation and to identify the species of root knot nematodes infesting the soil. *Meloidogyne incognita* (Koifoid et White) Chitw. was detected in Vittoria soil, while *Meloidogyne javanica* (Tremb.) Chitw. was detected at the Albenga and Caivano sites.

2.3. Telone C35 EC application

An emulsified formulation of the mixture 1,3-D/CP (Telone C35 EC—EF-1499; composition: 1,3-D 60.5% w/w, CP 33.3% w/w; $1.34\,kg\,l^{-1}$ density, Dow AgroSciences LLC, Indianapolis, IN, USA) was used. The fumigant was applied by drip fumigation at the application rates reported in Table 3. Drip lines (17 mm diameter) equipped with pressure compensating emitters (flow rate $2.4\,l\,h^{-1}$) were spaced 30 cm apart. 1,3-D/CP was injected into the irrigation line using a volumetric pump and adjusted to the required final concentration with different irrigation water amounts ranging from 15 to 45 mm to obtain a final chemical concentration in irrigation water of $900–1200\,mg\,l^{-1}$. At the Albenga site, 1,3-D/CP was drip applied on mechanically formed beds. Each bed (100 cm wide, 10 cm high) was provided with two drip lines, placed on the soil surface, 20 cm from the bed edge and 30 cm apart. The beds were spaced 100 cm apart. At the Caivano, Scoglitti and Acate sites, drip fumigation with 1,3-D/CP occurred on 2.5 and 4.0 m wide plots, respectively. Plots were provided with 5 and 8 drip lines placed on the soil surface at 25 cm from the plot edge and 50 cm apart. All trials included an untreated control and MB ($40\,g\,m^{-2}$, hot gas application; Metabrom980—composition: MB 98% w/w, CP 2% w/w, PM Chemicals—Italy) treated plots. Fumigants were applied under VIF film (Bromostop SIS®, IPM, Mondovì—CN, thickness 0.03 mm, permeability to $MB < 0.2\,g\,m^{-2} \times h$). After tarp removal, the soil was irrigated with at least 20 mm of water. At all sites, the VIF mulch was removed a few days before planting.

2.4. Tomato transplant and cultural practices

Tomato plants, directly sown in plastic trays into single cells of 33 mm of diameter and 40 mm of depth filled with a mixture of peat (50% vol/vol) and perlite (50% vol/vol), were transplanted into the fumigated beds and plots at the stage of two expanded leaves, 40–50 days after sowing. In Albenga, tomato plants (cv. Cuore di Bue, 66 plants $plot^{-1}$) were placed in two rows per fumigated bed. In Central and Southern Italy, tomato plants (Caivano: cv. Faino, 140 plants $plot^{-1}$; Scoglitti: cv. Jabot F_1, 120 plants plot^{-1}; Acate: cv Camelia F_1, 220 plants plot^{-1}) were transplanted in two and four rows, respectively. Plants were drip irrigated and grown following local commercial growers' cultural practices. After planting, four to six sprays with insecticides were applied at 7–10 day intervals to prevent the spread of viruses (tomato spotted wilt virus—TSWV—and tomato yellow leaf curl—TYLCV). Additional chemicals were preventively applied to reduce the risk of a sudden build-up of *Phytophthora infestans* and *Oidium neolycopersici* on leaves and *Botrytis cinerea* on stems and fruits.

2.5. Data collection and analysis

Disease development was evaluated during the cropping season at regular intervals by counting and removing infected plants showing severe decay symptoms. The number of healthy plants was also counted. Initially, plants showing wilting symptoms, basal and root rot were brought to the laboratory to identify the type of disease. Vascular wilt pathogens were detected using semi-selective media for fungi (Potato destrose agar amended with 100 ppm streptomicine sulfate) including Fusaria (Komada, 1975). Basal rot and root rot were detected using the same methods above. When Fusaria strains were isolated, a simple test on tomato seedlings (cv. Cuore di Bue) was performed as described by Jarvis and Thorpe (1976) to determine if they belonged to the forma specialis *F. oxysporum* f. sp. *radicis lycopersici*.

Nematode infestation was ranked according to a root knot galling index (0 = no galls at all; 1 = 1–5 small galls in one region of the root system; 2 = light attacks, no more than 20 galls spread across the root system; 3 = moderate attacks, plenty of small galls across the whole system; 4 = severe attacks with large galls reducing the size of the root system; 5 = very severe infestation with root system greatly reduced and deformed by a few large galls and lack of lateral roots) (Di Vito et al., 1979).

The root galling index was determined during the cropping season when plants were removed following the periodic disease assessments and at the end of the trials when all remaining plants were removed and the presence/absence of diseases/nematodes was carefully assessed.

The yield was determined by weighing the marketable fruits harvested from each plot. Data are expressed as average weight of marketable fruit per hectare. All yield data were transformed into an index where yield with MB was ranked as 100. Finally, all data collected were statistically analyzed using SPSS for Windows 12.0.1 software. Prior to analysis, data expressed as percentages were arcsine transformed to homogenize variances. Sources of variation were treatments and blocks. The effects of different fumigation treatments were examined using ANOVA and when the *F*-test was significant at $P<0.05$, treatment means were compared using the Tukey HSD test. The dose–response relationship between application rates of 1,3-D/CP and assessed responses (efficacy against pests and diseases, effect on yield) was analyzed by linear regression by performing a linear regression analysis. The influence of nematode infestation on soilborne disease infection was examined by calculating the Pearson's correlation coefficient.

3. Results

The untreated controls in trials 1 and 2 were heavily infested with *F. lycopersici* (Tables 3 and 4). In trial 1, in the untreated plots, the percentage of tomato plants affected by nematodes was 17.8%, nearly double the percentage of tomato plants affected by both fungi and nematodes (9.1%) (Table 3). Similarly, the percentage of tomato plants affected both by fungi and nematodes (9.1%) was nearly double the percentage of tomato plants affected only by fungi (5.1%, data not shown), thus confirming the high level of nematode infestation on the tomato crop (Table 3). In trial 2, in the untreated plots, the percentage of tomato plants affected both by fungi and nematodes (15.2%) was nearly 10 times higher than the percentage of tomato plants affected only by fungi (*F. lycopersici*, 1.6%) and, contrary to trial 1, the percentage of tomato plants affected by both nematodes and soilborne disease (15.2%) was higher than the percentage of tomato plants affected only by nematodes (6.0%), representing 66% of the total affected plants (Table 4).

Table 4
Percentage of tomato plants infected by *F. lycopersici*, *F. radicis-lycopersici*, *M. javanica*, *F. lycopersici* and nematodes, *F. radicis-lycopersici* and nematodes and in soil either untreated, or treated with 1,3-D/CP (C35) or MB (Trial 2, 2003—119 DAT)[a]

Treatment—rate g m^{-2}—amount of irrigation water	Only FOL[b]	Only nematodes	FOL + nematodes	FORL[c] + nematodes	Fungi + nematodes	Total
C35—26.84—30	7.6 a[d]	0.0 a	0.0 a	0.0 a	0.0 a	9.8 ab
C35—40.26—35	1.7 a	0.0 a	0.0 a	0.0 a	0.0 a	3.3 a
C35—53.68—45	2.2 a	0.0 a	0.0 a	0.0 a	0.0 a	2.7 a
MB—40	2.7 a	0.0 a	0.0 a	0.5 a	0.5 a	6.0 a
Control	1.6 a	6.0 b	12.0 b	3.3 b	15.2 b	22.8 b

For footnotes see Table 3.

In trials 1 and 2 soil fumigation reduced the number of plants infected by both Fusarium wilt (*F. lycopersici*) and galling nematodes; however, fumigant treatments were not significantly different from each other (Tables 3 and 4). Moreover, for plants infected only by *F. lycopersici*, no significant differences between MB, 1,3-D/CP mixture (Telone C35) and the untreated control were observed (Tables 3 and 4).

At the Albenga site, *F. oxysporum* f.sp. *radicis lycopersici* (FORL) spread homogeneously across treatments in trial 1 (Table 3) and in trial 2 (0.5–2.7%, data not shown). During trial 2, the control plots were significantly affected by the combined infestation of nematodes and FORL and no significant differences were found across the plots fumigated with 1,3-D/CP or MB (Table 4). The results were similar to those found for the effect of soil fumigation treatments on infestation by Fusarium wilt and galling nematodes: fumigation reduced the number of infected plants, however, fumigant treatments were not significantly different among each other.

Table 5
Percentage of tomato plants infected by *F. radicis-lycopersici* and by *Sclerotium rolfsii* in soil either untreated, or treated with 1,3-D/CP (C35) or MB (trials 3, 4, and 5, 2003)

Treatment—rate $g\,m^{-2}$—amount of irrigation water	Trial 3	Trial 4	Trial 5
	Scoglitti 149 DAT[a] FORL[b]	Acate 103 DAT FORL	Caivano 116 DAT *S. rolfsii*
C35—13.42—15	3.3 ab[c]	1.7 a	21.6 a
C35—26.84—30	5.4 b	2.2 a	13.6 a
C35—40.26—35	3.8 ab	3.2 a	11.8 a
C35—53.68—45	2.9 ab	3.5 a	22.3 a
MB—40	3.8 ab	3.5 a	10.5 a
Control	0.6 a	2.4 a	23.2 a

[a]DAT = days after transplant.
[b]FORL = *F. oxysporum* f.sp. *radicis lycopersici*.
[c]Numbers in the same column followed by the same letter are not significantly different according to Tukey HSD test ($P = 0.05$).

Trials 1 and 2 demonstrate the strong nematicidal effect of 1,3-D/CP, even when applied at low rates, and even on the combined infestation of fungi and nematodes (Tables 3 and 4). However, in trial 2, the percentage of plants infected by fungi and/or infested by nematodes (the total column) was lower when 1,3-D/CP was applied at 40.26 or 53.68 $g\,m^{-2}$ (Table 4).

Because the Vittoria area (Southern Italy) soils are extremely sandy (>90%) and historically infested with nematodes, tomato plants grown under commercial conditions were predominantly infested by galling nematodes and showed slight infections of FORL (Table 5). Tomatoes grown in the untreated plots had a high root galling index, but all 1,3-D/CP rates performed well (Table 6).

In trial 5, carried out in Central Italy, an unexpected heavy infestation of southern blight (*Sclerotium rolfsii* Sacc.) occurred after transplanting (Table 5). This pathogen is not commonly found in greenhouse crops in Italy, but is present on several vegetables such as tomato, sweet pepper, and green bean in Central and in Southern Italy (De Curtis et al., 2001). The southern blight infections occurred approximately 90 days after transplanting and then progressed randomly throughout the treated and untreated plots (Table 5).

Nematode infestation was evaluated at the end of all trials by calculating the root galling index. This index confirmed the nematicide activity of 1,3-D/CP (Table 6). The lowest rate of 1,3-D/CP was less effective, particularly in Southern (trials 3 and 4) and Central (trial 5) Italy. Moreover, in Central Italy (trial 5) the highest rate of 1,3-D/CP was significantly less effective than two of the lower rates. Finally, the galling indexes of the untreated plots in Central and Southern Italy (trials 3, 4 and 5) confirmed that nematodes are more problematic in those areas, most likely because of the soil texture (sandy) and the climate conditions (Table 6).

With regard to marketable tomato fruit weight, all rates of 1,3-D/CP resulted in an increase compared to the non-treated control and, in trials 1–4, to the plots treated with MB, too (Table 7). In trials 2–4 increases in marketable tomato fruit weight in plots treated with 1,3-D/CP

Table 6
Effect of soil disinfestation with 1,3-D/CP (C35) and MB on average root gall index (0–5) assessed at the end of the trial

Treatment—rate $g\,m^{-2}$—amount of irrigation water	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
	Albenga 79 DAT[a]	Albenga 129 DAT	Scoglitti 158 DAT	Acate 155 DAT	Caivano 126 DAT
C35—13.42—15	0.1 a[b]	–	0.3 a	1.0 b	2.4 b
C35—26.84—30	0.0 a	0.1 a	0.2 a	0.2 a	0.2 a
C35—40.26—35	0.0 a	0.1 a	0.1 a	0.4 a	0.5 a
C35—53.68—45	0.0 a	0.1 a	0.0 a	0.0 a	1.6 b
MB—40	0.0 a	0.0 a	0.0 a	0.2 a	0.3 a
Control	2.1 b	3.0 b	4.9 b	4.5 c	4.9 c

[a]DAT = days after transplant.
[b]Numbers in the same column followed by the same letter are not significantly different according to Tukey HSD test ($P = 0.05$).

Table 7
Efficacy of soil disinfestation with 1,3-D/CP (C35) on tomato yields

Treatment—rate g m^{-2}—amount of irrigation water	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
	Albenga 73 DAT[a]	Albenga 120 DAT	Scoglitti 158 DAT	Acate 155 DAT	Caivano 113 DAT
	Yield ha^{-1} (index: MB = 2.2 t)[b]	Yield ha^{-1} (index: MB = 11.7 t)	Yield ha^{-1} (index: MB = 10.4 t)	Yield ha^{-1} (index: MB = 45.1 t)	Yield ha^{-1} (index: MB = 38.5 t)
C35—13.42—15	122 a[c]	–	106 a	104 a	72 b
C35—26.84—30	123 a	81 a	91 a	103 a	86 ab
C35—40.26—35	105 b	102 a	115 a	97 a	88 ab
C35—53.68—45	98 b	107 a	115 a	99 a	82 b
MB—40	100 b	100 a	100 a	100 a	100 a
Control	58 c	37 b	42 b	76 b	43 c

[a]DAT = days after transplant.
[b]The tomato yield obtained from plots treated with MB, which is listed for each experiment, is ranked as 100.
[c]Numbers in the same column followed by the same letter are not significantly different according to Tukey HSD test ($P = 0.05$).

compared to plots treated with MB were not significantly different. However, for trial 1 where the marketable tomato fruit weight appeared very low, yields with the lowest rates of 1,3-D/CP were significantly different and higher than those treated with MB. For trial 5, where the southern blight infections caused severe damage, two rates of 1,3-D/CP provided yields that were not statistically different from those of MB, although they were lower (Table 7).

In the presence of severe *F. lycopersici* infestations (trials 1 and 2), the regression analysis showed significant relationships between the application rates of 1,3-D/CP (0–53.68 $g\,m^{-2}$) and the percentage of infected tomato plants, only in trial 2 (Table 8). In the presence of low disease incidence (trials 3 and 4) as well as with severe and homogenously widespread infections of *S. rolfsii* (trial 5), no significant relationship was observed between rates of 1,3-D/CP and the percentage of infected plants (Table 7). The rate–response relationship of 1,3-D/CP with the final nematode root index was inconclusive; it was not possible to relate the application rate and the concentrations ranging from 900 to 1200 $mg\,l^{-1}$ to the galling index (Table 8). Finally, positive and significant relationships were observed between rates of 1,3-D/CP and yield, but only for trial 2 (Table 8).

The evaluation of correlation between the total percentage of plants infected by *F. lycopersici* and the final root index describing the nematode infestations, showed, at the Albenga site, where the Fusarium wilt was constantly observed, the correlation between wilt disease incidence and *M. javanica* infestations (Table 9). Indeed, plants grown on plots treated with either MB or 1,3-D/CP had a low nematode infestation, certainly due to the 1,3-D activity in the 1,3-D/CP treated plots, and, in trials 1 and 2, a low wilt incidence (Table 9).

The same evaluation aimed at correlating the total percentage of plants infected by *F. radicis-lycopersici* and the final root index, did not shown correlations, except at Scoglitti site (Table 9).

4. Discussion

The results of our experiments as well as those of previous studies confirm that the mixture of 1,3-D 60.5% w/w and CP 33.3% w/w may be considered an effective fumigant on tomato (Csinos et al., 2000; De Cal et al., 2005; Gilreath et al., 1994, 2005; Gilreath and Santos, 2004; Locascio et al., 1997). However, fumigation efficacy depends on soil properties, amount of water used to apply fumigants by drip and plastic mulch (Ajwa et al., 2002; Desaeger et al., 2004; Gamliel et al., 1998; Minuto et al., 1999). Previous research in Italy showed that soil type and organic matter content may have a strong influence on the efficacy of treatments with CP alone, particularly when applied through shank injection (Gullino et al., 2002). In that study, the same application rate of CP was more effective in sandy soil (sand, 82%; silt, 7%; clay, 11%; pH 8.3; organic matter content, 0.7%; and cation exchange capacity, 5.0 meq 100 g $soil^{-1}$) than in sandy loam soils with a higher amount of organic matter (sand, 75%; silt, 20%; clay, 5%; pH 8.1; organic matter content, 2.5%; and cation exchange capacity, 8.5 meq 100 g $soil^{-1}$). Nevertheless CP delivery through drip irrigation systems reduced the influence of soil characteristics and the differences between MB and potential alternatives (Gullino et al., 2002) and the data collected in trials 1–4 support this previous findings: when root knot nematodes, *F. lycopersici* and/or *F. radicis lycopersici* were simultaneously observed, the analysis of variance, with experimental site location as the main factor, did not show significant differences on the basis of both nematode and disease incidence and root galling index (data not shown), thus excluding interactions between three experimental sites and 1,3-D/CP drip applied at 900–1200 $mg\,l^{-1}$. Nevertheless, our data did not permit

Table 8
Evaluation of rate–response relationship of 1,3-D/CP (C35) with the total percent of infected plants, the final nematode root index and the total yield throughout the analysis of regression and the estimation of R^2

Treatment—rate $g\,m^{-2}$—amount of irrigation water	Trial 1			Trial 2			Trial 3			Trial 4			Trial 5		
	% infected plants	Root index (0–5)	Yield $t\,ha^{-1}$	% infected plants	Root index (0–5)	Yield $t\,ha^{-1}$	% infected plants	Root index (0–5)	Yield $t\,ha^{-1}$	% infected plants	Root index (0–5)	Yield $t\,ha^{-1}$	% infected plants	Root index (0–5)	Yield $t\,ha^{-1}$
C35—13.42—15	4.7	0.1	2.7				3.3	0.3	11.0	1.7	1	46.9	21.6	2.4	27.7
C35—26.84—30	5.5	0	2.7	9.8	0.1	9.5	5.4	0.2	9.5	2.2	0.2	46.5	17.0	0.2	33.1
C35—40.26—35	3.5	0	2.3	3.3	0.1	11.9	3.8	0.1	12.0	3.2	0.4	43.7	11.8	0.5	33.9
C35—53.68—45	3.1	0	2.2	2.7	0.1	12.5	2.9	0	12.0	3.5	0	44.6	22.5	1.6	31.6
Control	32.0	2.1	1.3	22.8	3	4.3	2.5	5	4.4	8.2	4.5	34.3	46.6	5	16.6
R^2	0.560 NS**	0.536 NS	0.149 NS	0.947 *	0.771 NS	0.961 *	0.033 NS	0.551 NS	0.650 NS	0.233 NS	0.658 NS	0.286 NS	0.469 NS	0.512 NS	0.648 NS

*Significant linear regression ($P<0.05$).
**NS = non-significant linear regression ($P = 0.05$).

Table 9
Correlation between total percentage of plants infected by *F. lycopersici* or *F. radicis-lycopersici* and the final root index describing the nematode infestations

Treatment—rate $g\,m^{-2}$—amount of irrigation water	Trial 1			Trial 2			Trial 3		Trial 4		Trial 5	
	% plants infected by FOL	% plants infected by FORL	Root index	% plants infected by FOL	% plants infected by FORL	Root index	% plants infected by FORL	Root index	% plants infected by FORL	Root index	% plants infected by FORL	Root index
C35—13.42—15	2.3	2.3	0.1				3.3	0.3	1.7	1.0	0.2	2.4
C35—26.84—30	1.5	3.9	0.0	7.6	2.2	0.1	5.4	0.2	2.2	0.2	0.0	0.2
C35—40.26—35	1.5	2.0	0.0	1.7	1.6	0.1	3.8	0.1	3.2	0.4	0.0	0.5
C35—53.68—45	0.8	2.3	0.0	2.2	0.5	0.1	2.9	0.0	3.5	0.0	0.2	1.6
MB—40	3.8	2.3	0.0	2.7	3.3	0.0	3.8	0.0	3.5	0.2	0.0	0.3
Control	12.7	1.5	2.1	13.6	3.3	3.0	2.5	5.0	8.2	4.5	0.0	5.0
Pearson's coefficient	0.974	−0.544	–	0.888	0.503	–	−0.526	–	0.881	–	0.140	–
Significance of correlation	*	NS**	–	*	NS	–	NS	–	*	–	NS	–

*Significant correlation ($P<0.05$).
**NS = non-significant correlation ($P = 0.05$).

to relate the application rate and the concentration ranging from 900 to 1200 mg l^{-1} to soilborne disease and to the galling index, similarly to other experimental data obtained by drip fumigation with a similar emulsifiable mixture of 1,3-D and CP (InLine^{TM}), but at a concentration ranging from 1000 to 1500 mg l^{-1} (Desaeger et al., 2004).

Results at the Albenga site, infested with *F. lycopersici*, *F. radicis-lycopersici* and *M. javanica,* support previous findings of a correlation between wilt disease incidence and *M. javanica* (Back et al., 2002, Bergeson, 1972). Therefore, these results confirm the relationship between nematodes and wilt fungi infections (Back et al., 2002) and, particularly, in the host/disease complex tomato/*F. oxysporum* f.sp. *lycopersici*–*M. javanica* (Bergeson, 1972). Our experiments showed that the correlation within the disease complex *M. javanica*–*F. lycopersici* (trials 1 and 2) was more apparent than that between *M. javanica* or *M. incognita* and *F. radicis lycopersici* (trials 1–5).

None of the tested rates of 1,3-D/CP was effective against *S. rolfsii*, however, MB promoted a higher yield. More studies may be necessary on *S. rolfsii*, but our results underline some difficulties to control this pathogen with mixtures of 1,3-D and CP. Similar experiments might be necessary to determine the performance of 1,3-D/CP in soils infested with both galling nematodes and, for instance, corky root (*Pyrenochaeta lycopersici*), a common soilborne disease of greenhouse tomato.

In conclusion our experimental activities confirm the strong nematicidal effect of 1,3-D/CP without differences when drip applied at application rates ranging between 26.84 and 53.68 g m^{-2} or at the concentration ranging of 900–1200 mg l^{-1}. Moreover, even if studies conducted on strawberry grown in soils highly infested by soilborne pathogens and without relevant nematode infestation, demonstrate a significant increase in disease incidence when the rate of a similar emulsifiable mixture of 1,3-D and CP (InLine^{TM}) decreased from 393 to 236 l ha^{-1}, reducing the CP rate from 16.3 to 9.8 g m^{-2} (Ajwa and Trout, 2004), our results indicate that when the presence of plant parasitic nematodes enhance the incidence of Fusarium wilt of tomato, 1,3-D/CP effectively controls disease even at the lowest rate of 26.84 g m^{-2} (containing less than 9 g m^{-2} of CP).

Finally, it must be noted that mixtures of 1,3-D and CP can be combined with other control measures (Katan, 2000). Alternative fumigants perform better if the climatic conditions, soil composition, and the disease complex are fully known. For example, it has been shown that the ability of 1,3-D to control nematodes can be improved by increasing the soil temperature from 20 to 30 °C (Shikui et al., 2000). Moreover, organic matter content, soil moisture and plastic film (polyethylene vs. VIFs) are widely known to affect the diffusion and emission of 1,3-D, and thus its efficacy against soilborne nematodes. Besides higher sorption capacity, soils having a high organic matter content may reduce the efficacy of 1,3-D by promoting faster biodegradation (Thomas et al., 2003). Greater soil moisture can reduce the volatilization of 1,3-D and reduce its losses into the atmosphere, but the 1,3-D formulation may be diluted and/or affected in its fumigant potential and therefore may be less efficacious when fumigation is carried out in a saturated soil (Thomas et al., 2003). Therefore, soil moisture content must be carefully assessed before and after drip fumigation to optimize fumigant diffusion and efficiency in controlling fungi, nematodes and weeds, even if recent findings did not emphasize the effects of this factor particularly for nematode and weed control (Desaeger et al., 2004).

Finally, the possibility to broaden the spectrum of activity against weeds through application of mixtures of 1,3-D and CP by direct soil injection or drip irrigation with metham sodium has to be carefully considered, particularly because of recent findings concerning the incompatibility of halogenated fumigants with metham sodium (Guo et al., 2005). Nevertheless, inadequate weed control represents a risk, particularly in fields infested by *Cyperus* spp. (Gilreath and Santos, 2004); this weed can harbor and protect *M. incognita,* limiting the nematicidal efficacy of 1,3-D (Thomas et al., 2004) and, on the basis of our results, probably enhances the infection of Fusarium wilt in tomato fields.

5. Conclusions

In the presence of pest and disease complexes of root knot nematodes (*M. incognita*, *M. javanica*, *F. lycopersici* and *F. radicis lycopersici*) and without the significant presence of weeds, the drip application of the water emulsifiable mixture of 1,3-D 60.5% w/w and CP 33.3% w/w under gas-tight films results in satisfactory pest and disease control on greenhouse tomatoes in Italy. In sandy soils (Southern Italy), with slight *F. radicis lycopersici* infections and with heavy nematode (*M. incognita*) attacks, Telone C35 EC, drip applied at 900 mg l^{-1} during late summer (fumigation: late summer; transplant: late-summer/autumn; last harvest: early spring), performs well up to 132.4 kg ha^{-1} (100 l ha^{-1}).

In sandy loam soils (Northern Italy) with slight *F. radicis lycopersici* infections and severe infections of *F. lycopersici* and galling nematodes (*M. javanica*), 268.4 kg ha^{-1} (200 l ha^{-1}) of 1,3-D/CP drip applied at 900 mg l^{-1} provided yields similar to those of MB treated plots both in spring and summer cycles. In sandy loam soils in Central Italy with severe infections of *S. rolfsii*, our results are inconclusive to identify an effective rate of 1,3-D/CP applied before the tomato spring season. Additional research is necessary to determine the long-term efficacy of treatments with mixtures of 1,3-D and CP in Italy, the possible beneficial effects of supplementary/subsequent applications of fumigant and non-fumigant pesticides on soilborne pest control and tomato yield across more than one cycle and the performance against problematic pests (root knot nematodes), diseases (Fusarium wilt and crown and root rot causal agents, *S. rolfsii*) and weed (*Cyperus* spp.) complexes.

Moreover, because our evaluations, in terms of disease and nematode control and of tomato yield, show that increased rates of the emulsifiable mixture 1,3-D (60.5% w/w) + CP (33.3% w/w) drip applied does not always produce improved outcomes, further experiences are needed in order to better understand the interaction in soils drip fumigated with nematicide and fungicide mixtures aimed at optimizing low and effective rates of 1,3-D/CP for Italian protected tomatoes.

Acknowledgments

The authors thank Paris Tsakonas for the helpful suggestions in reviewing the manuscript; Laura Gaggero and Mattia Sanna for the work in processing data; Carlo Spotti, Emanuele Nobile and Stefano Ardente for the organization of the trials and for the cooperation in the data collection for disease, nematode and yield evaluations; Maria Immacolata Coiro and Antonio Carella for the technical support given during the research on nematodes; and the farmers for the kind support during the field trials.

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