Control of *Pyrenochaeta Lycopersici* on tomato by ozone disinfestation

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Abstract

The control of corky root on tomato caused by *Pyrenochaeta lycopersici* has been generally based on soil fumigation with methyl bromide. The use of this product is banned from 2005 because of its heavy environmental effect. The objective of this research was to determine and demonstrate the efficacy of soil treatment with ozone in the control of corky root on tomato in greenhouse and in open field, as alternative to methyl bromide. In the trial carried out in greenhouse, ozone, produced by an ozone generator, was applied into the soil by drip sub irrigation (20 cm) using drip lines equipped with water emitters (4 l/h) every 30 cm. The ozone was applied as gas in moistened soil or dissolved in irrigation water. In the open field trial the ozone was applied dissolving it in irrigation water by drip sub irrigation with drip lines set on the mulched soil. Plots were distributed in a randomized block design with four replications for each treatment. Untreated plots were used as control. In both trials severity of corky root was significantly reduced in ozone treated plots compared to control, either on main and secondary roots. In open field trial a significant difference was found between the two ozone treatments, as severity of corky root in plots treated by drip irrigation was higher than sub irrigation treated plots. In the two experiments all ozone treatments significantly increased tomato marketable yield compared to untreated control.

Key-words: Corky root, *Pyrenochaeta lycopersici*, ozone, soil disinfestation

Introduction

Intensive and repeated planting of tomato (*Lycopersicon esculentum* Mill.) either in open field and in protected crop has increased the severity of corky root caused by *Pyrenochaeta lycopersici* Schneider and Garlach (Ciccarese *et al*., 1993) (Fig. 1). In particular, control of this soilborne pathogen, as for other pathogens, weeds and phytoparasitic nematodes, has been generally based on chemical treatments, especially soil fumigations with methyl bromide. The use of this product has been banned since the beginning of 2005 because of its heavy environmental effects on stratospheric ozone depletion. A series of other chemicals, is also available in different liquid or granular formulations (fumigants and non fumigants), many of which may be banned after the revision of the European Community (Basile *et al*., 2003). In every case the increasing attention to the environment safety and to the human health, require to find new alternative control strategies, environmentally sound and economically convenient at the same time.

During the last decade research on low environmental impact alternatives have received a strong impulse and considered a wide range of options, including agronomic (green manures, amendments, crop rotations) and physic methods (soil solarization and steam), the use of biocidal plants, biological control agents and resistant tomato cultivars (Minuto *et al*., 1995; Gamliel *et al*., 2000; Tamietti and Valentino, 2000; Tjamos *et al*., 2000; Sasanelli and Greco, 2000; Vannacci and Gullino, 2000).

Ozone treatments of soil may represent a potential alternative to the use of chemicals for the control of many soilborne pathogens including *P. lycopersici*.

Therefore, two trials were carried out to verify the possibility of ozone treatments for the control *P. lycopersici* on tomato either in greenhouse and in open field.

Fig. 1. Tomato roots affected by Pyrenochaeta lycopersici

Materials and methods

Greenhouse.

The trial was performed in a greenhouse naturally infected by *P. lycopersici* at Valenzano (province of Bari, Apulia region, southern Italy) in the experimental farm of the University of Bari. The soil was deeply ploughed, rotavated and subdivided in 12 m^2 (3 x 4m) plots, spaced 1,2 m each other, and distributed in a randomized block design with four replicates per treatment. The ozone was applied either alone as gas into moistened soil or dissolved in the irrigation water. Ozone gas, produced on site with an ozone generator, was injected before transplanting into the soil through drip tubing of 16 mm PVC tubes with water emitters every 0.3 m. The drip tubing was buried just next the transplanting rows, at a depth of 0.2 m in the soil. Untreated and dazomet treated plots were used as controls. Tomato seedlings, cv. Super Marmande, were transplanted in each plot in three rows spaced 0.8 m. Seedlings were spaced 0.3 m along the row.

Open field.

A medium-textured soil at Valenzano heavely infested by *P. lycopersici* was deeply ploughed, rotavated and subdivided in 50 m^2 plots, spaced 1,2 m each other, and distributed in a randomized block design with four replicates for each treatment. Two irrigation systems (drip irrigation and subirrigation) were performed to allow different ozone treatments. The ozone was applied, by drip and subirrigation (depth 20 cm), in each plot by six PVC drip lines (∅ 16 mm) equipped with water emitters (flow rate 4 l/h) every 30 cm. Drip irrigation lines were covered with black plastic mulch (polyethylene, 40 µm thickness). This covering system was also used in all other plots of the experiment (Fig. 2). Ozone dissolved in irrigation water (0.8 mg O_3/m^3) was produced in site by an ozone generator for 12 hours. Ozone untreated plots were used as control applying the same amount of water irrigation.
One month old seedlings of tomato (*Lycopersicum esculentum* Mill.), cv. Caspar, were transplanted in the plots, two days later ozone treatments, in three coupled rows spaced 0.8 m and seedlings were spaced 0.6 m along the row.

In both trials during the growing season the plants received the usual cultural practices. At the end of the crop cycle in each plot tomato marketable yield and root weights were recorded. Severity of corky root on main and secondary roots was estimated according to a scale of six classes from 0 to 5 (0 = root health and 5 = > 75 % affected root surface).

All data from both experiments were statistically analysed by analysis of variance (ANOVA) and means compared by Duncan's Multiple Range Test (SAS – 1997).

Fig. 2. View of the field experiment with the sub-irrigation system and plots covered with black plastic mulch (polyethylene, 40 m thickness).

Results and discussion

Greenhouse.

In the greenhouse experiment ozone, as gas formulation, and dazomet treatments significantly increased the number of fruit and tomato marketable yield per plant in comparison to the untreated control. Only the ozone treatment in which the gas was dissolved in the water irrigation was not significantly different from the untreated control (P=0.01) (Tab. 1).
All ozone treatments and dazomet significantly reduced severity of corky root either on main and secondary roots in comparison to untreated control. Severrity of corky root on main roots was significantly lower in ozone gas treatments than those observed in ozone dissolved in water irrigation and dazomet treatments (P=0.01) (Tab. 2).

Table 1. Effect of different ozone treatments on tomato in greenhouse.

Treatment	Dose g/m²	Mode of application	Marketable yield /plant g/plant			N° fruit/plant		
Ozone	20 – 1 day before transplanting	Gas 1 time	1837*	a**	A	14.3	a	A
Ozone	20– 1 day before transplanting	Gas 2 times	1854	a	A	13.5	a	A
Ozone	20– 1 day before transplanting	Gas dissolved in water irrigation	1676	ab	AB	13.1	ab	AB
Dazomet	50 – 30 days before transplanting		1842	a	A	14.4	a	A
Untreated control			1535	b	B	11.7	b	B

* Each value is an average of four replications;
** Data flanked in each column by the same letter are not statistically different according to Duncan's Multiple Range Test (small letters for P=0.05; capital letters for P=0.01).

Table 2. Effect of different ozone treatments on severity of corky root on main and secondary tomato roots in greenhouse conditions.

Treatment	Severity of corky root					
	Main root			Secondary root		
Ozone gas (1 time)	1.3*	a**	A	1.3	a	A
Ozone gas (2 times)	1.6	ab	A	1.6	ab	B
Ozone in water irrigation	1.9	b	B	1.6	ab	B
Dazomet	2.0	b	B	1.8	b	B
Untreated control	3.1	c	C	3.0	c	C

* Each value is an average of four replications;
** Data flanked in each column by the same letter are not statistically different according to Duncan's Multiple Range Test (small letters for P=0.05; capital letters for P=0.01).

Open field.

In the field experiment ozone treatments significantly increased tomato marketable yield and root weight compared to the untreated control. Application of ozone by subirrigation resulted better than drip irrigation treatment (Tab. 3).

Severity of corky root was significantly reduced either on main and secondary roots, compared to control, in ozone treated plots (Tab. 4). A significant difference was found also between the two ozone treatments, as severity of corky root in plots where ozone was applied by drip irrigation was higher than subirrigation.

The higher efficacy of subirrigation treatment could be due to the direct activity of ozone in the soil layer including tomato roots. Increase marketable yield in ozone treated plots is explained by reduction of severity of disease and beneficial effect of ozone on the soil fertility (Fig. 3).

Potential benefits of soil ozone treatment concern: a) no transportation, storage, discharge of hazardous or toxic chemicals; b) no chemical residues; c) minimum human acute and chronic toxicity and d) no human carcinogenicity or teratogenicity. Therefore, ozone treatments could be considered as a valid alternative to the use of methyl bromide, which use is banned in Italy and treatments by subirrigation should be favourably considered for tomato protection either in open field and in greenhouse conditions against *P. lycopersici* and, very likely, against other soilborne pathogens and parasites. Moreover, by this method it is possible to reduce production costs for the repeated use of the irrigation system in several cultural practices (irrigation, fertilization, and crop protection).

Table 3. Effect of ozone treatment on root weight and marketable yield of tomato plants cv. Caspar growth in naturally Pyrenochaeta lycopersici infested field.

Treatments	Marketable yield (q/ha)		Root weight (g/root system)	
Ozone - drip irrigation	213.6*	A**	107	AB
Ozone – subirrigation	273.6	B	119	B
Control (untreated)	160.8	C	91	A

* Each value is an average of four replications;
** Data flanked in each column by the same letter are not statistically different according to Duncan's Multiple Range Test (P=0.01).

Table 4. Effect of ozone treatment on severity of corky root caused by Pyrenochaeta lycopersici on tomato roots (cv. Caspar).

Treatments	Severity of corky root			
	Main root		Secondary root	
Ozone - drip irrigation	2.4*	A**	2.6	A
Ozone - subirrigation	1.7	B	2.2	B
Control (untreated)	4.0	C	4.2	C

* Each value is an average of four replications (each of them formed by twenty samples);
** Data flanked in each column by the same letter are not statistically different according to Duncan's Multiple Range Test (P=0.01).

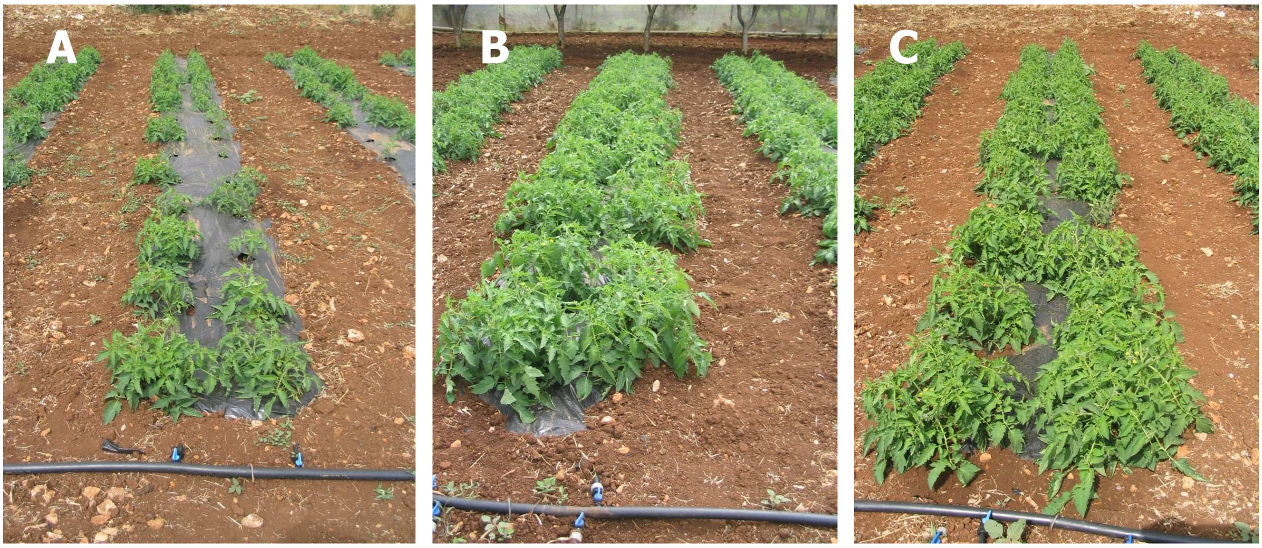

Fig. 3. Plots: A - Untreated; B – Ozone applied by subirrigation; C – Ozone applied by drip irrigation

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