DOI 10.2478/s11687-008-0027-y

HELMINTHOLOGIA, 45, 3: 137 – 142, 2008

Aphanocladium album by via sub-irrigation in the control of *Pyrenochaeta lycopersici* and *Meloidogyne incognita* on tomato in a plastic-house

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Summary

Two experiments were carried out to assess the efficacy of different chemicals (azoxystrobin, fosthiazate, metham-sodium) and of the chitinolytic fungus *Aphanocladium album* (isolate MX-95), that could be alternatives to methyl bromide, against the soil borne pathogen *Pyrenochaeta lycopersici* and the root-knot nematode *Meloidogyne incognita* on tomato in a plastic house in southern Italy. In the first trial, the treatments were azoxystrobin (1.25 l a.i. /ha), fosthiazate (1.5 l a.i. /ha) and biological control agent *Aphanocladium album* isolate MX-95 (2.5 l/plot at $2x10^7$ CFU/ml; plot surface 96 m^2). In the second experiment, treatments were metham-sodium (1000 l c.p./ha) and *A. album* (5 l/plot at $1x10^7$ CFU/ml). In both trials, chemicals and the fungus were applied by via sub-irrigation. Satisfactory control of the corky root and the root-knot nematode attack and a significant yield increase were obtained by application of azoxystrobin, fosthiazate and metham-sodium. A significant reduction of *M. incognita* soil population density occurred in plots treated with *A. album*. Also, high positive correlations were found between the symptoms caused on tomato roots by *M. incognita* and *P. lycopersici*.

Key words: Biological control; corky root; root-knot nematodes; chemical treatments; sub-irrigation

Introduction

A number of studies on the interaction between phytoparasitic nematodes and other soilborne pathogens, both on vegetables and fruit trees, have been conducted (Abawi & Chen, 1998; Ciccarese *et al.*, 2001a; Francl & Wheeler, 1993; Lamberti *et al.*, 2001a). During the last decade, severe corky-root symptoms, caused by *Pyrenochaeta lycopersici* Schneider *et* Gerlach, have frequently been found in association with attacks by *Meloidogyne* spp. (Polizzi *et al.*, 2004), both under field and protected conditions in tomato-growing areas. Generally, control of these and other soilborne pathogens was based on chemical treatments, especially soil fumigations with methyl bromide. However, the use of this product has been banned since the beginning of 2005 in developed countries because of its stratospheric ozone depletion. Therefore, its utilization is limited only for "critical use".

A series of other chemicals, is also available as liquid or granular formulations (fumigants and non fumigants), but many of them could be banned after the revision of the European Community (Reg. EC 414/91) (Basile *et al.*, 2003). Moreover, increased concerns for environment safety and human health are stimulating investigation to find new and alternative control strategies that are environmentally sound and economically convenient.

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 & Valentino, 2000; Tjamos *et al.*, 2000; Sasanelli & Greco, 2000; Vannacci & Gullino, 2000; Sasanelli *et al.*, 2002; D'Addabbo & Sasanelli, 2003; Nico *et al.*, 2004; D'Addabbo *et al.*, 2005; Castillo *et al.*, 2006).

Recently a new promising biological control agent *Aphanocladium album* (Preuss) W. Gams isolate MX-95 that is antagonistic to nematodes, fungi and insects (Biali *et al.*, 1972; Yaniv *et al.*, 1979; Ciccarese *et al.*, 2007) could represent a new possible alternative method control against phytoparasitic nematodes and soilborne pathogens to the use of chemicals, thanks to its intensive chitinolytic activity (Ambrico *et al.*, 2002).

Therefore, two trials were carried out on tomato in pro-

tected crops to investigate i) the suitability of a new technology to apply different treatments (chemical and biological) at lower environmental impact, and that could be alternative to methyl bromide, and ii) the interaction between the soil-borne pathogen *P. lycopersici* and the root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw.

Materials and Methods

First trial

A plastic-house of 2.173 m^2 (41 x 53 m) at Leverano (province of Lecce, Apulia region, southern Italy), with sandy soil heavily infested by *M. incognita* (9 eggs and juveniles/cm^3 soil) and *P. lycopersici,* of which severe symptoms were evident in the previous tomato crop cycles, was selected. The soil was deeply ploughed, rotavated and subdivided in 96 m^2 plots (8 x 12 m), spaced 1 m apart, and distributed according to a randomized block design with four replicates per treatment. A sub-irrigation system (depth 20 cm) was performed in each plot by PVC drip lines (∅ 1.6 cm) equipped with water emitters (flow rate 4 l/h) every 30 cm to allow different treatments (Fig. 1).

Fig. 1. Sub-irrigation system with PVC drip lines (∅ 1.6 cm) equipped with water emitters (flow rate 4 l/h) every 30 cm

Treatments were: a) azoxystrobin (1.25 l a.i./ha, corresponding to 5 l c.p./ha); b) fosthiazate (1.5 l a.i./ha, corresponding to 10 l c.p./ha) and c) *Aphanocladium album* isolate MX-95 (biological control) (2.5 l/plot of a conidial suspension at $2x10^7$ CFU/ml). Untreated plots served as controls.

In each plot, tomato seedlings (cv. Luisa) were transplanted in 3 coupled rows spaced 1 m each other and with 0.8 m between the rows of the same coupled row. Plants were spaced 0.3 m along the row.

Azoxystrobin was applied at transplanting whereas fosthiazate and *A. album* were applied 1 and 3 days before transplanting, respectively. The chemicals, as liquid formulation, and the fungus *A. album* were applied by the sub-irrigation technique connected to a tank, in which were dissolved the products and in which was inserted an aspiration pump (Fig. 2).

A. album was reared *in vitro* (Fig. 3). The mycelium was dissolved in sterile water and sown in PDA Petri dishes

Fig. 2. Distribution system of agro-chemicals and biological control agent by sub-irrigation

incubated in the dark for 7 days at 24 °C. The mycelium was then homogenized in sterile water with a tensioactive to allow the dispersion of strongly hygroscopic conidia. Then, the concentration of the inoculum was determined and diluted to obtain $2x10^7$ CFU/ml standard conidial suspension. The suspension was then applied by sub-irrigation at a dose of 2.5 l/plot. A filter was set to avoid at the beginning of the sub-irrigation system blocking of the irrigation system.

Fig. 3. *Aphanocladium album* isolate MX-95

During the growing season the tomatoes received the cultural practices that are common for the area like weed, insect and pathogen control, fertilizer application, irrigation and use of useful pollinators (bumblebees).

Tomatoes were harvested six times from 20 July to 20 October and the total marketable yield of the entire crop cycle calculated. Plants from the central coupled row in each plot were uprooted to estimate the root gall index caused by the nematode attack according to a 0-5 scale (0 no galls and 5 root system completely deformed by large and numerous galls) (Lamberti, 1971). The severity of corky root on main and secondary roots was estimated also according to a 0-5 scale (0 = root healthy; 1 = 1-10% affected root surface (a.r.s.); 2 = 11-25% a.r.s.; 3 = 26-50% a.r.s.; 4 = 51-75% a.r.s. and 5 = > 76% a.r.s.). Nematodes were extracted from soil samples of each plot processing 500 cm^3 soil sub-sample with the Coolen's method (Coolen, 1979).

Second trial

After one year, the same plastic house was used for a second experiment as above indicated. Plots not treated in

the previous year were used in this experiment (4.7 eggs and juveniles/cm^3 soil).
The soil was deeply ploughed, rotavated and subdivided in 96 m^2 plots (8 x 12 m), spaced 1 m apart, and distributed according to a randomized block design with four replicates per treatment. The sub-irrigation system was performed as previously described.
On the basis of results from the first crop cycle treatments were: a) metham-sodium (470 Kg a.i./ha) and b) *A. album* isolate MX-95 (5 l/plot of a conidial suspension at 1x10^7 CFU/ml) applied two times. Untreated plots were used as controls.
Tomato seedlings of cv. Luisa were transplanted in each plot as in the first experiment and during the growing season plants were treated as in the previous year.
Metham-sodium and *A. album* were applied one month before transplanting. One month after transplanting the *A.album* treatment was repeated at the same concentration. The fungus was reared and conidial suspension prepared as mentioned in the first experiment. Tomatoes were harvested 6 times from 20 May to 25 July and the total marketable yield calculated.
Plants from the central coupled row in each plot were uprooted to estimate the root gall index and the severity of corky root on main and secondary roots according to the different 0-5 scales previously described. Nematodes were extracted from each plot as in the first trial.
In both trials data were subjected to analysis of variance (ANOVA) and means compared by Duncan's Multiple Range Test.

Results and Discussion

First trial
Azoxystrobin was the only treatment that increased significantly tomato marketable yield in comparison to untreated control. No statistical differences were observed in comparison with the other two treatments (P = 0.05) (Tab. 1). With this treatment, symptoms and severity of corky root were also significantly reduced either on main and secondary roots (P = 0.01).

Table 1. Effect of azoxystrobin, fosthiazate and *Aphanocladium album* treatments on marketable yield of tomato (cv. Luisa) in a plastic-house infested with *Pyrenochaeta lycopersici* and *Meloidogyne incognita*

Treatment	Dose	Application time	Marketable yield (q/ha)		
Control (untreated)	0	0	436*	a**	A
Azoxystrobin	5 l .p./ha	At transplanting	498	b	A
Fosthiazate	10 l c.p. /ha	1 day before tranplanting	470	ab	A
Aphanocladium album MX-95	2,5 l (2x10^7 CFU/ml)	3 day before tranplanting	447	ab	A

*Average of four replications; **Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (small letters for P = 0.05; capital letters for P = 0.01

The nematicide fosthiazate was more effective than azoxystrobin and *A. album* to reduce gall index and final soil *M. incognita* population (P = 0.05) (Tab. 2).
The nematode soil population density was also significantly suppressed by *A. album* isolate MX-95 (P = 0.01) (Tab. 2), thus suggesting that it is more effective than the antagonistics *Streptomyces griseoviridis* strain K61 and *Trichoderma harzianum* strain T-22 tested previously on tomato under protected crop conditions (Filippi *et al.* 2001; Percoco & Amenduni, 2001), similar to those of our experiment and with the contemporary presence of *P. lycopersici* and *M. incognita*. However, the root gall index on the tomato roots in plots treated with *A. album* MX-95 was not reduced. This can be explained by the enzymatic chitinolytic activity of the fungus that reaches its maximum about 3 weeks after the application of the fungus (Ciccarese *et al.*, 2001b; Ambrico *et al.*, 2002).

Second trial
Metham-sodium significantly increased tomato marketable yield in comparison to untreated control. No statistical difference was observed between *A. album* treatment and

Table 2. Effect of azoxystrobin, fosthiazate and *Aphanocladium album* treatments against *Pyrenochaeta lycopersici* and *Meloidogyne incognita* on tomato (cv. Luisa) in a protected crop

Treatment	*P. lycopersici*						*M. incognita*					
	Infestation index (0 – 5)						Gall index (0 – 5)			Final population (eggs and juveniles /cm^3 soil)		
	Main root			Secondary root								
Control (untreated)	1.8*	a**	A	2.5	a	A	4.0	a	A	35	a	A
Azoxystrobin	1.0	b	B	1.6	b	B	2.9	b	B	25	b	AB
Fosthiazate	0.6	c	B	1.4	b	B	0.4	c	C	5	d	C
A. album isolate MX-95	1.8	a	A	2.4	a	A	3.9	a	A	15	c	BC

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

the other treatments (metham-sodium and untreated control) (P = 0.01) (Tab. 3). Moreover, metham-sodium treatment significantly decreased root gall index and severity of corky root either on main and secondary roots because of its fungicide and nematicide activity due to the development of methylisothiocyanate.

Table 3. Effect of the metham-sodium and *Aphanocladium album* treatments on marketable yield of tomato (cv. Luisa) in a plastic-house infested with *Pyrenochaeta lycopersici* and *Meloidogyne incognita*

Treatment	Dose	Application time	Marketable yield (q/ha)		
Control (untreated)	0	0	360*	a**	A
Metham-Na	1000 l c.p./ha	1 month before tranplanting	720	b	B
A. album isolate MX-95	5 l (1x10^7 CFU/ml)	1 month before and after transplanting	490	a	AB

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

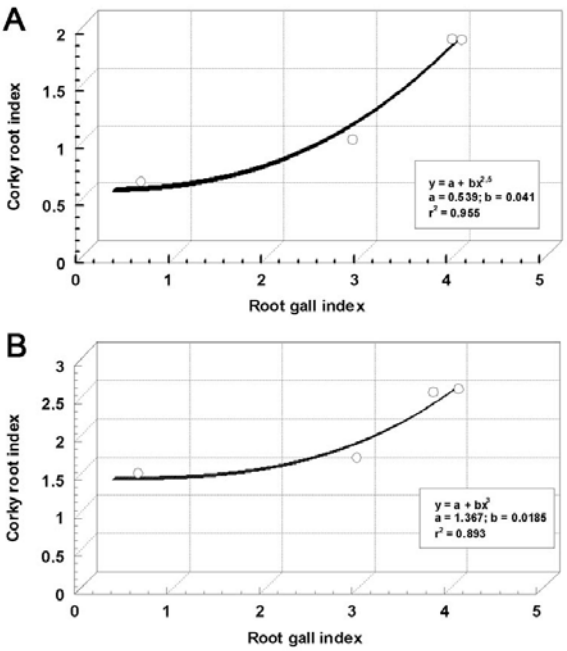

Fig. 4. (I trial) Relationship between gall index, caused by *M. incognita*, and severity of corky root on main tomato roots (A) and on secondary tomato roots (B) caused by *P. lycopersici*

The nematode soil population density and the root gall index were also significantly suppressed by *A. album* isolate MX-95 (P = 0.05) (Tab. 4), confirmed the results from the first experiment.

The observed reduction of root gall index may be attributed to the number and time of application of the fungus. In fact, in this second experiment *A. album* was applied two times: one month before transplanting and one month later. So in this way the fungus had the possibility to develop its maximum enzymatic chitinolytic activity protecting the seedlings at transplanting from the nematode attack. The second fungus application served as post-transplant protection during the crop cycle. The *A. album* treatment did not reduce infestation index of *P. lycopersici* on secondary roots, whereas it was effective on main root with a significant reduction of severity of symptoms (Tab. 4).

The experiments revealed significant and positive correlations between root gall index and the severity of corky root on main (r^2 = 0.955 and 0.975) or secondary root (r^2 = 0.893 and 0.851) (Figs. 4 and 5). Therefore, it seems that an increase of the nematode attack increases also the severity of *P. lycopersici*. Probably the trophic activity of the second juvenile stage of *Meloidogyne* on the roots of tomato through the mechanical action of the stylet, that open new wounds, favours penetration and infection of the root tissue by the soil born pathogen *P. lycopersici* (OEPP/EPPO, 2004). This mechanism would explain why the severity of corky root on the tomato roots was not suppressed by *A. album* during the first trial.

Therefore, the low values of gall index observed on the tomato roots in azoxystrobin, fosthiazate and metham-sodium treated plots would explain the lower severity of symptoms of corky root in the same plots. Infestation indexes of *P. lycopersici* were significantly higher on the

Table 4. Effect of the metham-sodium and *Aphanocladium album* treatments against *Pyrenochaeta lycopersici* and *Meloidogyne incognita* on tomato (cv. Luisa) in a protected crop

Treatment	*P. lycopersici*						*M. incognita*					
	Infestation index (0 – 5)						Gall index (0 – 5)			Final population (eggs and juveniles /cm^3 soil)		
	Main root			Secondary root								
Control (untreated)	1.7*	a**	A	2.8	a	AB	3.7	a	A	18	a	A
Metham-Na	0.6	b	B	1.7	b	B	0.5	c	C	4	b	B
A. album isolate MX-95	1.1	b	B	3.4	a	A	2.0	b	B	10	b	AB

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

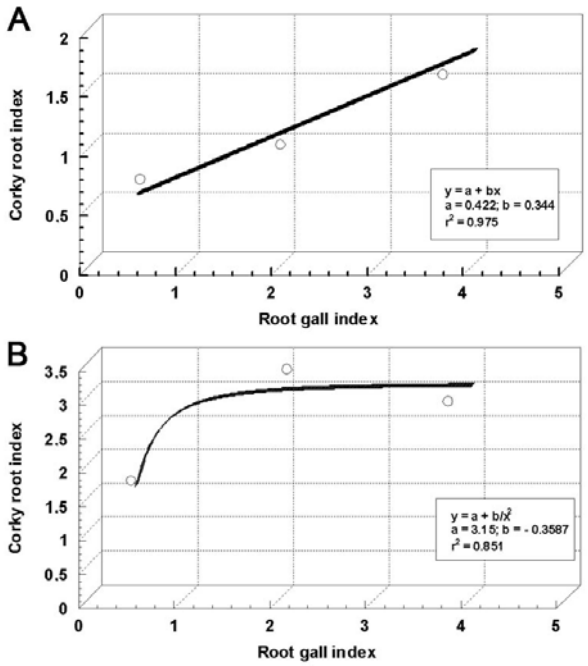

Fig. 5. (II. trial) Relationship between gall index, caused by *M. incognita*, and severity of corky root on main tomato roots (A) and on secondary tomato roots (B) caused by *P. lycopersici*

roots treated with azoxystrobin (chemical with fungicide activity) than that of roots treated with fosthiazate (chemical with nematicide activity). This is explained by the more opened ways to the penetration of the pathogen fungus that the azoxystrobin is unable to contain because it has not nematicidal activity (Tab. 2). In fact, the fosthiazate and the metham-sodium carring out an intense nematicidal activity are able to protect the root systems from *M. incognita* juvenile attacks giving consequently less possibilities of penetration and infection of *P. lycopersici*.

Conclusions

Azoxystrobin, fosthiazate and metham-sodium should be recommended for the control of simultaneous attacks of *P. lycopersici* and *M. incognita*. For these chemicals and also for biological products application through the sub-irrigation technique would results more beneficial than with any other methods of aplication. In fact, in this way it is possible to reduce production costs because of the repeated use of the irrigation system in several cultural practices (irrigation, fertilization, and crop protection).

Among crop protection methods with low enviromental impact, soil solarization is positively considered to control both soil borne pathogen and root-knot nematodes but its use is not always possible and convenient (Aloj *et al.*, 1998; Lamberti *et al.*, 2001b). Soil solarization requires covering the soil for a long time (1-2 months) with costly plastic materials, can be performed only in warm areas and for high value crops (Garibaldi & Gullino, 1991). Application of *A. album* isolate MX-95 has no enviromental impact and therefore could be considered routinely for the control of these soil borne pathogens and nematodes if it is applied more than 1 time and at least 1 month before transplanting. However, more investigations are suggested to ascertain the most appropriate rates and timing of application.

Aknowledegement

The research was undertaken within the framework of a bilateral project between the National Council of Research (CNR-Italy) and the Slovak Academy of Science (SAV-Slovak Republic). Project N° 3/CNR 3 (2007-2009).

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RECEIVED FEBRUARY 8, 2008

ACCEPTED JULY 23, 2008