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***CURRICULUM*: "CONTROL WITH MINIMUM ENVIRONMENTAL IMPACT"**

INTEGRATED AND SUSTAINABLE CONTROL OF COLLAPSE OF MELON IN CENTRAL ITALY

by

Roberto Reda

Coordinator

Prof. Leonardo Varvaro

Supervisor

Prof. Gabriele Chilosi

Assistant Supervisor

Prof. Paolo Magro

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Integrated and sustainable control of collapse of melon in Central Italy

Abstract

The collapse of melon caused by a complex of fungal pathogens, including *Monosporascus cannonballus,* is one of the most destructive diseases worldwide. The goal of this research was to investigate integrated and sustainable control strategies against *M. cannonballus* in Central Italy. More specifically, we aimed to investigate occurrence of melon collapse in greenhouse grown and to assess control strategies against *M. cannonballus*. Moreover we aimed to observe the effects of compost amendment on sudden wilt and the influence of the endomycorrhizal fungus *Glomus intraradices* (AFM) on the development of *M. cannonballus*. The results confirm that the *M. cannonballus* occurrence ranged from 24% to 30% of all the farms surveyed and that the measures built up in melon producing area in Province of Viterbo are un-sustainable and effective only by few tools such as fumigation and grafting on squash. Experiments under greenhouse conditions have shown that compost amendment is capable to reduce the severity of collapse caused by *M. cannonballus* on melon. Moreover, inoculation with AMF alone is not sufficient for the complete prevention melon collapse. Control of melon collapse can be obtained with a sustainable and integrated strategy promoting fertility maintenance and restoration of soil health.

Keywords: melon collapse; *Monosporascus cannonballus*; integrated disease management; compost; *Glomus intraradices*; sustainable control.

Controllo integrato e sostenibile del collasso del melone nel Centro Italia

Riassunto

Il collasso del melone, causato da un complesso gruppo di patogeni fungini, tra cui *Monosporascus cannonballus*, è una delle malattie più distruttive in tutto il mondo. L'obiettivo di questa ricerca è stato quello di studiare strategie di difesa integrata e sostenibile contro *M. cannonballus* nel Centro Italia. Quindi è stata verificata la presenza di *M. cannonballus* su melone coltivato in serra e sono state valutate le strategie di difesa del territorio. Inoltre sono stati valutati gli effetti del compost sul collasso del melone e l'influenza del fungo endomicorrizico *Glomus intraradices* (AFM), sull'azione di *M. cannonballus*. I risultati hanno confermato che nel territorio la presenza di *M. cannonballus* è compresa tra il 24% ed il 30% delle aziende monitorate e che le uniche strategie di difesa efficaci sono la fumigazione e l'innesto su zucca. Inoltre l'applicazione del compost in serra può ridurre l'incidenza del collasso, mentre la micorrizazione delle piante con *G. intraradices* da sola non riesce a limitare l'azione di *M. cannonballus*. Il controllo del collasso del melone può essere ottenuto quindi integrando strategie di difesa sostenibili che possano ripristinare e mantenere la fertilità biologica del suolo.

Parole chiave: collasso del melone; *Monosporascus cannonballus*; difesa integrata; compost; *Glomus intraradices;* controllo sostenibile.

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General introduction

Soil-borne fungal diseases that affect cucurbits have a worldwide distribution, and have become limiting factors for growing in many major areas producers.

The disease known under the name 'Melon collapse', also referred to as 'sudden death', 'sudden wilt' or 'root rot and vine decline', is an aetiology complex disease which has become prominent in all the muskmelon production in Italy (Chilosi *et al.,* 2008) and generally worldwide (Martyn and Miller, 1996). Collapse of melon is characterised by the sudden and generally uniform canopy collapse just prior to harvest, resulting often in the total fruit loss (Martyn and Miller, 1996; Cohen *et al*., 2000). The disease is considered particularly severe in the hot arid and semi-arid production regions of the world (Martyn and Miller, 1996; Aegerter *et al*., 2000; Cohen *et al*., 2000).

Melon production

Melon (*Cucumis melo* L.) is annual trailing vine-plants belonging to the Cucurbitaceae family. At the worldwide level, melon cultivation covers approximately 1.000.000 hectares. In Tables 1.1 is listed the production data and acreage for melon in 2008 in major producing countries. China is the main melon producer, covering more than 14 million tons, which represents near the 52% of the world's production. The major European producers are Spain, France and Italy. In Italy melon crops cover are approximately 26.000 hectares (12% in protected crops and the rest in fields), providing a production of approximately 650.000 tons. The main Italian regions producers are Sicily, Emilia-Romagna, Lombardy

and Latium (Table 1.2, Sources ISTAT, 2009).

In Italy, the cultivated melons may be essentially divided into three groups:

1. *Cantaloupensis*: the "Cantaloupe melons", including fruits of average size with a smooth surface, which may be stored only briefly and include varieties such as Charentais.
2. *Inodorus*: the "winter melons", having medium or big fruit, with smooth white rind and flesh white or greenish. This group is mainly grown in southern regions in open field, where hot and dry environment improves sweetness and shelf life of fruits.
3. *Reticulatus*: the "netted melons", including medium-sized fruit with thin reticulated rind and sweet orange flesh. Since many of these crops come from the United States, they are also known as American melons. In Italy, this group is grown in greenhouse and in open field and includes cultivars Proteo (Syngenta Seeds), reference variety for Italian market.

Table 1.1 Production data and acreage for melon in major producing countries (Sources: FAO, 2008)

Countries	Area (ha)	Production (tons)	%
China	570.874	14.322.480	51,82
Turkey	105.000	1.749.935	6,33
Iran	80.000	1.230.000	4,45
USA	35.990	1.042.530	3,77
Spain	33.388	1.021.800	3,70
Egypt	31.500	757.677	2,74
Morocco	31.255	736.800	2,67
Italy	28.199	653.309	2,36
India	24.011	645.000	2,33
Mexico	23.472	582.288	2,11
Guatemala	18.900	445.035	1,61
Brasil	15.746	340.464	1,23
France	14.747	265.576	0,96

Table 1.2 Melon crop cover and production in Italy (Sources: ISTAT 2009).				
	Open field		Greenhouse	
Regions	Area (ha)	Production (tons)	Area (ha)	Production (tons)
Piedmont	305	8.516	3,53	135
Lombardy	1.991	57.104	714,8	24.760
Liguria	2	24	-	-
Veneto	1.225	37.159	407,47	14.492
Friuli-V. Giulia	1	20		
Emilia-Romagna	1.487	37.117	329,65	6.879
Tuscany	789	18.124	49	1.951
Umbria	351	10.540	-	-
Marche	231	6.365	2,43	85
Latium	985	28.620	430,04	16.161
Abruzzo	428	13.180	2,5	80
Molise	40	320	-	-
Campania	699	30.629	579,5	19.899
Apulia	2.340	57.700	65	2.150
Basilicata	671	13.343	300	7.288
Calabria	958	27.415	24,42	1.345
Sicily	9.160	161.509	280,35	23.834
Sardinia	1.069	18.008	20,22	1.751
ITALY	**22.732**	**525.695**	**3.208,91**	**120.814**

Melon soil-borne diseases

Fungal diseases that affect roots on cucurbits have a worldwide distribution, becoming limiting factors for growing in many major producing areas. Among them, particularly damaging is the diseases known under the name "vine decline" (Bruton, 1998). Most of the soil-borne diseases consist of a complex of several pathogens interacting with the predominant pathogen and contributing to the disease syndrome.

In Table 1.3 are reported the major melon soil-borne diseases worldwide according to the classification proposed by Bruton (1998).

Table 1.3 Major melon soil-borne diseases worldwide		
DISEASE	**PATHOGEN**	**SIMPTOMS AND SIGNS**
Charcoal rot	*Macrophomina phaseolina* (Tassi) Goid.	Water-soaked green stem lesion. Gumming on stems. Small, black structures (pycnidia) in lesions.
Damping-off	*Pythium* spp. *Rhizoctonia solani* Kühn	Water-soaked lesions on roots and stem. Wilting, death.
Gummy stem blight	*Didymella bryoniae* (Auersw.) Rehm.	Water-soaked lesion on leaves. Gummy stem lesions.
White mold	*Sclerotinia sclerotiorum* (Lib.) de Bary	Soft, watery rot on stems and fruits In contact with soil. White mycelium and black sclerotia.
Fusarium wilt	*Fusarium oxysporum* Schlechtend f. sp. *melonis* Leach & Currence	Dark brown lesions on one side of stem. Brown discoloration of vascular tissue. Pink micelium in lesions.
Verticillium wilt	*Verticillium dahliae* Kleb. *V. albo-atrum* Reinke et Berth.	Wilting death. Brown discoloration of vascular tissue.
Collapse of melon (sudden wilt)	*Monosporascus cannonballus* Pollack & Uecker	Root rot, vine collapse. Attacks primary and secondary roots, especially at the juncture of the roots. Black, round structures (perithecia) on root death.
	Acremonium cucurbitacearum A. Alfaro-Garcia, W. Gams, et J . Garcia-Jimenez *Rhizopycnis vagum* D.F. Farr *Plectosporium tabacinum* (van Beyma) M.E. Palm, W. Gams et Nirenberg.	Root rot, vine collapse. Lesion at the stem—root junction, discoloration of roots, death of secondary and tertiary roots.

Collapse of melon

The symptoms of this syndrome are similar to those described for other vine decline diseases (García-Jiménez *et al.*, 2000). These symptoms include the yellowing and death of the crown leaves and a gradual decay of the vine, as the fruits approach maturity (Fig. 1.1). The fruits of affected plants are small in size, have a low sugar content and are more prone to sunburn (Mertely *et al.,* 1991; Martyn and Miller 1996). Aboveground symptoms are visible just prior to harvest resulting in rapid wilt of plants, premature fruit ripening and low sugar content of fruits. The typical symptoms of the disease are localised on root systems (Fig. 1.2), represented by root lesions, root rots and loss of smaller feeder roots (Chilosi *et al.*, 2008). The collapse is a complex of diseases of melon characterised by similar

symptomatology, but different aetiology (Abad *et al.*, 2000). *Monosporascus cannonballus* Pollack & Uecker and *Acremonium cucurbitacearum* Alfaro-Garcia, W. Gams & García-Jiménez have been reported as the primary causal agents of this disease (Martyn and Miller, 1996; Cohen *et al.*, 2000; García Jiménez *et al.*, 2000). Other species associated with the disease are *Rhizopycnis vagum* D.F. Farr and *Plectosporium tabacinum* (van Beyma) M.E. Palm, W. Gams et Nirenberg (Abad *et al.*, 2000; Armengol *et al.*, 2003).

In Italy, the disease has been recorded on both melon and watermelon in the main growing areas since 1999 (Gennari *et al.*, 1999; Infantino *et al.*, 2002, 2004; Montuschi, 2002; Stravato and Vagnozzi, 2003; Buzi *et al.*, 2004).

The pathogens which are associated with this disease are *M. cannonballus* in Central Italy, *R. vagum* and *M. cannonballus* in Northern Italy, *A. cucurbitacearum* and *P. tabacinum* in Southern Italy (Chilosi *et al.*, 2008).

Fig. 1.1 Collapse of melon in greenhouse grown.

Fig. 1.2 Typical symptoms of root rot lesion.

Monosporascus cannonballus Pollack & Uecker

Occurrence

M. cannonballus has host range within the Cucurbitaceae, but its hosts display different degrees of susceptibility (Mertely *et al.*, 1993a). Muskmelon and watermelon have been classed as susceptible or highly susceptible. All the

cucurbits tested were susceptible to *M. cannonballus*. The pathogen was readily isolated from both infected watermelon and muskmelon plants, whereas it was difficult to recover from Cucurbita species. However, it was demonstrates that the pathogen is capable of infecting the tissues of a species which is resistant (autumn squash) and a species which is susceptible (muskmelon) to melon collapse (Alfaro-Fernández and García-Luis, 2009). Therefore, while the growth of watermelon and muskmelon plants was severely affected, *Cucurbita* species were less stunted and there was a smaller reduction in their maximum weight (Mertely *et al*., 1993a).

M. cannonballus, was described as a *genus et species novus* by Pollack and Uecker in 1974 based on specimens obtained from necrotic muskmelon roots from Arizona. Hawksworth and Ciccarone (1978) obtained a strain of this fungus, isolated from *Triticum* sp. from Libya. The first confirmed report of pathogenicity was from Israel in 1983 where it was shown to the cause of a mature melon plant collapse, although the pathogen was identified as *M. eutypoides* (Reuveni and Krikun, 1983). An earlier report from Japan indicated that this fungus was nonpathogenic (Uematsu *et al.,* 1985). Pathogenicity of isolates from the United States was first reported in 1991 by Mertely *et al.* in Texas, and the disease was named *Monosporascus* root rot and vine decline. Up to that date, the disease was monitored in various countries. In Eastern Spain, this syndrome appears to be associated in most cases with two fungal agents: *A. cucurbitacearum* (Alfaro-Garcia *et al.,* 1996; Armengol *et al*., 1998) and *M. cannonballus* (Lobo Ruano, 1990). Mixed infections are frequently found in this area (Martyn and Miller, 1996). *A. cucurbitacearurn* was also associated with melon vine decline in California (Bruton *et al*., 1995) and Texas (Bruton *et al*., 1996). *M. cannonballus* has also been reported as a causal agent of severe root rot of muskmelon known as '*Monosporascus* root rot/ vine decline', economically damaging in North America (Martyn *et al*., 1991; Mertely *et al*., 1991; Stanghellini *et al*., 1996), Israel (Reuveni and Krikun, 1983; Pivonia *et al.,* 1997), and Japan (Uematsu, 1991). Additionally, this fungus was investigated as a possible causal agent of muskmelon vine decline in Central America (Martyn *et al*., 1996; Bruton and Miller, 1997a,b), South Eastern

and Western Asia (Tsay and Tung, 1995; Martyn and Miller, 1996; Karlatti *et al.*, 1997), and Northern Africa (Martyn et *al.,* 1994). The last countries that have been described for the first time are Korea (Kwon *et al.*, 2001), Brasil (Sales *et al.*, 2004), and, recently, Iran (Sarpeleh, 2008).

In Italy, first isolated in watermelon (Gennari *et al.*, 1999), later in melon (Infantino *et al.*, 2002b) and cucumber (Montuschi, 2002).

The identities of the most representative fungal pathogens in Italy in the early years when they appeared signs of "collapse" are reported in Table 1.4.

Table 1.4 Identities of the most representative fungal pathogens causing collapse of melon in Italy (Sources: Chilosi *et al.*, 2008).

Regions	Pathogens	Years
Emilia-Romagna	*R. vagum*	2001
	M. cannonballus	2006
Lombardy	*M. cannonballus*	2003
Latium	*M. cannonballus*	2005
Apulia	*A. cucurbitacearum* *P. tabacinum*	2002

Taxonomy and description of *M. cannonballus*

M. cannonballus is a homothallic pyrenomycete forming large (40-50 μm-diam), spherical, multi-layer walled ascospores; however typically only one ascospore is formed per ascus instead of the usual eight. Ascospores are multinucleate, typically having eight nuclei, although occasionally they may have 16. There is no known anamorph (asexual) stage (Martyn, 2002). Perithecia are formed in host root tissue and *in vitro* on artificial growth media (Fig. 1.3). Ascospores are thick (Fig. 1.4), multi-walled spores and are extremely resistant to desiccation and other factors (Martyn and Miller, 1996; Stanghellini *et al.,* 1996). *In vitro* vegetative growth is optimal at 25 to 35°C, while perithecia are formed most readily at 25 to 30°C. The fungus may survive for several days at temperatures up to 55 C°, but is killed within 90 min at 60°C. Mycelial growth occurs over a pH range of 5 to 9, but is

optimal from pH 6 to 7 and inhibited completely at pH 4 and below. *M. cannonballus* appears also to be adapted to slightly or moderately alkaline and saline soils (Kwon *et al.*, 2001). The fungus grows readily on several standard laboratory growth media (e.g. potato dextrose agar, V-8 juice agar, and water agar) and forms fertile black perithecia within 2 to 3 weeks. Perithecia are readily visible against the light gray or dirty white mycelium.

In culture (e.g., PDA, V-8 juice agar, water agar), wild-type *Monosporascus* colonies typically are a dirty-white or light grey color with numerous black perithecia embedded in the agar. Under some circumstances, colonies may produce pigments giving the mycelia a yellow to orange to dark brown colour. Pigmented colonies are slow-growing, produce few, if any, perithecia, and are often hypovirulent (see below).

Fig. 1.3 *M. cannonballus*: myceliar growth and perithecia formed in host root tissue *in vitro* on artificial growth media.

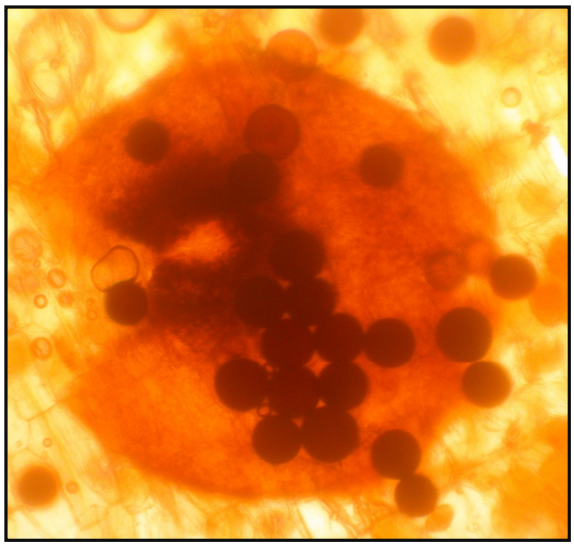

Fig. 1.4 Ascospores of *M. cannonballus*.

Disease cycle

Climate

The rapidity and severity of collapse are generally associated with high temperatures level late in the growing season (Wolff, 1996; Wolff *et al.*, 1997;

Bruton *et al*., 1999; Pivonia *et al*., 2002b) and heavy fruit loads (Wolff, 1996; Pivonia *et al*., 1997; Wolff *et al*., 1997; Pivonia *et al*., 2002a). Daily maximum/minimum air temperatures of 35/20 °C near the end of the growing season were associated with vine decline (Bruton *et al*., 1999). It is also found a highly correlation between soil temperatures above 20 °C during the first 30 days after planting and the incidence of vine decline at the end of the season (Pivonia *et al*., 2002b). Heating of soil during the winter cropping season resulted in more rapid disease development than in no heated soil (Pivonia *et al*., 2002b). Conversely, it was found that a low temperature regime after transplanting can stress plants during early root development, delaying growth; therefore, fruits mature under higher temperatures more conducive to *M. cannonballus* (Aleandri *et al*., 2010). Kim *et al* . (1995) reported that soil temperatures of 25 °C or greater for 8 or more consecutive h per day were required for initial root infection.

Inoculum

Ascospores, which are produced in perithecia on infected roots (Fig. 1.5), function as the primary survival structure and inoculum (Stanghellini *et al*., 1996; 2000).

Fig. 1.5 *M. cannonballus*: perithecia in melon infected roots.

The ascospores are released into the soil from the perithecia when the infected roots start to decompose (Pollack and Uecker 1974; Martyn and Miller 1996; Cohen *et al*., 2000) and are able to survive in the soil in the absence of muskmelon production, even under flooding conditions (Beltrán *et al*., 2005). It has been demonstrated that pathogen reproduction occurs primarily at the end of the growing season (Waugh *et al*., 2003; Stanghellini *et al*., 2004a; Boughalleb *et al*., 2010). Waugh *et al.* (2003) noted that the root system of a single mature cantaloupe plant is capable of supporting the production of approximately 400.000 ascospores. Consequently, the fungus has great potential to maintain and/or increase in affected melon fields.

Ascospores of *M. cannonballus* have been recovered from commercial muskmelon fields that have been surveyed by different researchers. Waugh *et al.* (2003) indicated that known commercial cantaloupe problem fields contain as few as 2 ascospores/g of soil. This value is not noticeable different from those reported from commercial muskmelon fields in south Texas (Mertely *et al*., 1993b), Arizona and California (Stanghellini *et al*., 1996, 2004a,b; Aegerter *et al*., 2000; Waugh *et al*., 2003; Radewald *et al*., 2004), Korea Republic (Heo *et al*., 2001), Spain (Beltrán *et al*., 2005; 2007), Tunisia (Boughalleb *et al*., 2010). It was found that in muskmelon and watermelon, a decrease of ascospore counts in soil from transplanting until first symptoms of vine decline (Beltrán *et al*., 2008). These authors observed a remarkable increase of ascospore counts in a parallel way to the quick development of vine decline symptoms. In contrast, there were no symptoms of vine decline on grafted watermelon without perithecia formation, and stable ascospore soil populations. It was suggested by authors that disease control by grafting onto genus *Cucurbita* can be related primarily by the increased resistance of its root system to infection by *M. cannonballus*.

Ascospores can germinate readily in the rhizosphere of the host plants growing in field soil. Germination is encourage by the production of root exudates, however, it was found that also that the soil microflora may be involved in the induction of

ascospore germination (Stanghellini *et al*., 2000, 2010).

Germ tubes of ascospore germlings are firmly anchored to melon roots, but no structures resembling appressoria can be observed; the tips of germ tubes, upon contact with the epidermis, appears to penetrate the epidermis directly (Waugh *et al*., 2001). Mycelial growth during the initial phase of root colonization is mainly radial growth through the cortex and establishment of the fungus within the xylem, much like a vascular wilt pathogen (Waugh *et al*., 2001). However, unlike true vascular wilt pathogens such as *Verticillium dahliae* and *Fusarium oxysporum, M. cannonballus* does not produce secondary spores to aid its subsequent movement in plant.

Using light and electron microscopy, Waugh *et al.* (2005) observed that germ tubes penetrates the epidermis, and hyphae grows, without branching, almost directly to the xylem. Than hyphae traverses the endodermis into protoxylem cells, and grow extensively within the lumen of metaxylem vessels. *M. cannonballus* therefore appears to be most similar to vascular wilt pathogens in its mode of parasitism, but does not spread via the vascular system to above-ground plant tissues.

In a study aimed to compare the response of *C. melo* and *C. maxima* to infection by *M. cannonballus* in terms of colonisation and histological changes it was found that the xylem vessel lumina of both muskmelon and autumn squash showed hyphae and tylose formation as a result of both fungal infections (Alfaro-Fernández and Amparo García-Luis, 2009). However, non-fungal structures were detected in the hypocotyl vascular tissues. Authors demonstrated that both fungi are capable of infecting the tissues of a species which is resistant (autumn squash) and a species which is susceptible (muskmelon) to melon collapse. Extensive tylose formation has previously been described in *Monosporascus*-infected muskmelon (Alcántara *et al*., 1998; Pivonia *et al*., 2002). These structures may reduce water flow in plant vessels and may be the cause of wilting, particularly under conditions where water demand is high (Pivonia *et al*., 1997).

M. cannonballus colonisation is presumably mediated by the production of cellulosolytic and among pectolytic enzymes, by polygalacturonase and metyl-

pectin esterase (Gregori *et al*., 2007).

Symptoms

Plants affected by this disease suffer root damage (discoloration, brownish lesions, and necrosis) in the taproot and secondary roots from the early growth stages, which results in a loss of their water uptake capacity (Fig. 1.6). This deterioration of the radical system leads to a gradual vine yellowing and decay, often resulting in total collapse as the plant approaches fruit maturity, the period of maximum demand for water (Fig. 1.7). As a consequence a high percentage of fruit do not reach commercial size, have low sugar content, and suffer from sun scald, resulting in most cases in a total loss of the crop (Mertely *et al*., 1991; Martyn and Miller, 1996).

Fig. 1.6 Discoloration, brownish lesions and roots necrosis caused by *M. cannonballus*.

Fig. 1.7 Collapse of melon plant before the harvest.

Control

Control of collapse of melon has proven to be difficult. At present, there is no one method available that is both cost effective and long lasting that provides adequate

control of the disease, although research using several different strategies is ongoing in several laboratories.

As described by Martyn (2002) factors contributing to the difficulty in controlling vine decline include:

1. intensive and successive cropping of melons, allowing for the build-up of inoculum;
2. presence of numerous thick-walled ascospores that may reside in the soil for years and which are apparently tolerant to desiccation and chemicals;
3. the general difficulty in applying and incorporating pesticides into the soil environment;
4. the inherent buffering capacity of the soil and plant rhizosphere;
5. the apparent wide host range of the fungus and its ability to colonize and reproduce on several Monocotyledons and Dicotyledons plants;
6. cropping practices such as plastic mulch and buried drip irrigation that create a favorable soil environment for infection and survival of the fungus;
7. cultural practices that result in poorly developed and shallow root systems;
8. lack of melon genotypes that are resistant or highly tolerant to infection and disease.

In spite of these obstacles, progress is being made in managing this disease. An integrated various approach to the disease management may be the best strategy. Integration increases the chance of developing effective management programs by combining partially effective methods, reducing the chances of negative side effects, and providing flexibility in adapting the control programs to different agricultural situations. Despite this, a full control of the disease in greenhouse, where symptoms of the disease is severe, is problematic in practice. For this reason, more tools for a sustainable management of the disease is strongly needed.

Chemical soil treatment.

The most effective technique so far has been preplant soil fumigation with methyl bromide, chloropicrin, 1,3 dichloropropene, or metam sodium. Preplanting soil disinfestation with methyl bromide, a common treatment for disease management, has been banned in many countries; other compounds have been applied experimentally via drip irrigation and have shown various degrees of control (Stanghellini *et al.*, 2003). Soil fungicide application during the growing season is one possible treatment. Fluazinam applied experimentally via drip irrigation during the season effectively controlled disease in Israel during the spring planting, but was less effective in the late-summer crop (Cohen *et al*., 1999); however, fluazinam is not labeled for use on cucurbits in outers countries at USA and Italy. Fludioxinil is registered for control of *M. cannonballus* on melons and watermelons when applied via drip irrigation in USA (Syngenta, 2008).

In Israel, also the fungicides azoxystrobin, prochloraz and pyraclostrobin + boscalid exhibited high and similar efficacies in the control of sudden wilt disease under field conditions (Pivonia *et al*., 2010).

A multi-phased strategy consisting of a preplant soil fumigation to reduce the resident inoculum in the soil, a postplant application of fungicide to inhibit root colonization during the season, and a postharvest cultivation of plants along with an immediate application of metam sodium to prevent ascospore production has been developed for control of vine decline in California (Martyn, 2002). Soil fumigation is recognize as a not GAP practice, since it is lethal not only for the pathogens, but also for the microbial beneficial microbes, thus abating the microbiological component of soil fertility.

Soil solarization.

Solarization of soil and soil-less media is a nonchemical approach to pest disinfestation that in the appropriate situations can be comparable to methyl bromide fumigation (Conventional soil solarization has been found to be ineffective in controlling of collapse of melon, presumably due to the ability of the

fungus to grow at high temperatures. However, in Israel a modified method of solarization in open containerized plants and the combination of soil solarization with reduced rates of fumigation have shown good potential for control of *M. cannonballus* (Cohen *et al.*, 2000; Pivonia *et al.*, 2002c).

Genetic resistance.

Like most soil-borne diseases, genetic resistance to collapse of melon is the method of choice for control; however, a good source of resistance to *M. cannonballus* has not yet been identified. Few melon lines have been identified to be tolerant to *M. cannonballus*, including several accessions of *C. melo* subsp. *agrestis* and several Galia and Ananas-type melons (Iglesias *et al.*, 2000; Dias *et al.*, 2004; Fita *et al.*, 2007). Root structure is believed to be a major factor in the grafting of susceptible melons onto rootstock of *Cucurbita* spp. and gourds for control of vine decline (Cohen *et al.*, 2005; Alfáro-Fernandez and García-Luis, 2009).

Grafting.

Grafting is a suitable method to control soil-borne diseases in melon crops (Trionfetti-Nisini *et al.*, 2002; Cohen *et al.*, 2007; Louws *et al.*, 2010). In the case of collapse, susceptible cultivars can be grafted onto *Cucurbita* spp. or *Lagenaria* spp. rootstock. This practice is used most commonly in Asia and the Mediterranean basin for the control of several soil-borne diseases, primarily *Fusarium* wilt, but increasingly for management of collapse of melon (Cohen *et al.*, 2005; Alfáro-Fernandez and García-Luis, 2009). There is generally good rootstock-scion compatibility among many of the cucurbit species, and the large and extensive root systems of these winter squashes and gourds allow them to survive infection during the season. The use of these rootstocks should be approached with some caution. Prolonged use of resistant rootstocks could encourage the spread of virulent pathogens.

Biological control

Fungal antagonists

Use of microbial antagonists in biocontrol of melon fungal disease caused by *M. cannonballus* is not clear. According to several publications, laboratory experiment using *Trichoderma* ssp. (Zhang *et al.*, 1999; Khalifa *et al.*, 2008) and *Chetomium* spp. (Sales *et al.*, 2007) suggest that biocontrol is a strategy for the integrated management of melon collapse. Reda *et al.* (2008) isolated from non-cultivated soil beneficial bacteria that able to inhibit growth of *M. cannonballus* and induce resistance in melon.

Hypovirulence

The use of hypovirulent isolates of *M. cannonballus* was investigated as a biological control for collapse of melon. During a survey in fields in the Lower Rio Grande Valley of Texas it was revealed that approximately 65% of the *M. cannonballus* isolates recovered from diseased plants harbored dsRNAs (Lovic, 1994). It was observed that after laboratory maintenance, most dsRNAs isolates developed a degenerate culture phenotype characterized by slow growth, yellow to orange pigment accumulation, and reduced ascospore production in culture (Lovic, 1994; Martyn and Miller, 1996). In addition, these dsRNA+ isolates were hypovirulent on muskmelon in greenhouse pathogenicity trials. Several experiments involving curing dsRNA+isolates and transferring dsRNAs to wildtype dsRNA−isolates suggested that some dsRNAs were causing the debilitating phenotypes (Park, 1996). Wheeler *et al.* (2004) reported that degenerate isolates from California, Texas, Honduras, Israel, and Spain did not produce melanin as opposed to wild type isolates. They further postulated that the loss of fungal melanization in *M. cannonballus* may be associated with loss of virulence in the pigmented isolates. In a farther study, a collection of isolates were grouped based on dsRNA fragment

sizes using cluster analysis and Euclidean distances (Cluck *et al*., 2009). Three distinct dsRNA groupings were observed. Group 2 isolates containing 2, 3, and 3.5 kb dsRNA appeared to exhibit a decrease in perithecia production compared to the other groups. Group 1 isolates exhibited yellow pigmentation only, while Group 3 isolates expressed grey (wild-type) and yellow (degenerate) pigmentation. Isolates that did not contain dsRNA (Group 4) exhibited wild-type pigmentation. The degenerative effects of dsRNA in *M. cannonballus* have been explored as a potential biocontrol strategy by Batten *et al*. (2000).

Resistance inducers

Treatment of melon seeds with methyl jasmonate, a known inducer of pathogen defense mechanisms in plants, reduced the severity of melon collapse under experimental conditions and offers an additional potential control strategy in the future (Reda *et al*., 2008; Aleandri *et al*., 2009, 2010).

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CHAPTER 2

Research objective

The general goal of this thesis research was to investigate integrated and sustainable control strategies against *M. cannonballus*. More specifically, we aimed to:

1. investigate occurrence of collapse of melon in Central Italy in greenhouse grown to confirm disease soil-borne fungal pathogens;
2. assess control strategies against collapse of melon in Central Italy to verify efficacy;
3. study the effects of compost amendment on melon collapse severity;
4. investigate the influence of the endomycorrhizal fungus *Glomus intraradices* on the development of collapse of melon by *Monosporascus cannonballus*.

CHAPTER 3

Occurrence of collapse of melon in Central Italy during 2008-2010

Roberto Reda

Department of Plant Protection – Tuscia University – Viterbo - Italy

ABSTRACT

Melon represents the most widespread cucurbit crop in the producing area of Central Italy. In recent years melon has been subjected to significant losses in yield and quality due to an increasing number of soil-borne fungal diseases. The collapse of melon, caused by a complex of fungal pathogens, including *Monosporascus cannonballus,* represents one of the most destructive disease worldwide. The purpose of this study was to determine the occurrence of collapse throughout melon-producing areas in greenhouse in a location in Central Italy, to verify the identification of isolates collected, and evaluate the putative relationship among disease and cultural practices, with particular attention to varietal reaction. In one hundred farms surveyed, the *M. cannonballus* occurrence monitored during the period 2008-2010 ranged from the 24% (2008), to 30% (2009) and 28% (2010) of total farms surveyed. Early varieties and melon rootstocks were found highly susceptible, late varieties were more tolerant whereas squash genotypes were full resistant.

Keywords: *M. cannonballus*, survey, resistance, monitoring, symptomatology

INTRODUCTION

Melon (*Cucumis melo* L.) represents one of the most economically important vegetable crops in Italy covering approximately 26.000 ha, principally in open field (22.720 ha) and in greenhouse (3.200 ha) (Istat, 2009). Production is obtained most commonly in three producing areas, North (27%), Central (14%) and Southern Italy (59%). In Central Italy, Latium is the most important regions with 1.400 ha (34% in greenhouse), mostly diffused in coastal areas.

Intensive horticulture in the coastal area of North Latium is basically associated to two main reasons: small farm and specialised production (greenhouse grown). Intensive agriculture requires less land than an extensive agriculture farm to produce a similar profit; because of these conditions, soil-borne diseases are one of the main limiting factors in crop production.

In Latium, melon cultivated in greenhouse recently has been subjected to significant losses in yield and quality due to an increasing number of soil-borne diseases of fungal origin affecting the root system (Buzi *et al*., 2004; Reda *et al*., 2005, 2008a, 2008b; Reda and Chilosi, 2006; Chilosi *et al*., 2008). In most cases symptoms on affected plants are similar and aetiology is difficult to confirm. *Fusarium oxysporum* f. sp. *melonis* and *Verticillium dahliae* have been implicated in melon wilt (Buzi *et al*., 2002; Infantino *et al*., 2004). Symptoms include the wilting of stems and foliage and vascular discoloration of roots. *F. oxysporum* f. sp. *melonis* race 1,2 has been reported as the most frequent pathogenic fungi in many Italian melon growing areas (Tamietti *et al*., 1994, Belisario *et al*., 1998). *Rhizoctonia solani* AG4 have been also recorded in Central Italy as a destructive causal agent of root rot and wilting of cantaloupe (Corazza *et al*., 1992). *Sclerotinia sclerotiorum* occurs on melon roots, stems and fruits in greenhouse determining severe losses in seasons characterised by low temperature during spring (Chilosi *et al*., 2004).

The collapse is a complex of diseases of melon characterised by similar symptomatology, but different aetiology (Abad *et al*., 2000) and represents one of the most destructive soil-borne disease of this crop worldwide. *Monosporascus cannonballus* and *Acremonium cucurbitacearum* have been reported as the primary

causal agents of this disease (Martyn and Miller, 1996; Cohen *et al*., 2000; García Jiménez *et al*., 2000). Other species associated to the disease are *Plectosporium tabacinum* and *Rhizopycnis vagum* (Abad *et al*., 2000; Armengol *et al*., 2003). Foliage and fruits symptoms are visible just prior to harvest resulting in rapid wilt of plants, premature fruit ripening and low sugar content of fruits. Specific symptoms of the disease are localised on root system and are represented by root lesions, root rots and loss of smaller feeder roots. In Italy, the disease has been recorded on both melon and watermelon in the main growing areas since 1999 (Gennari *et al*., 1999; Infantino *et al*., 2002, 2004; Montuschi, 2002; Stravato and Vagnozzi, 2003; Buzi *et al*., 2004; Chilosi *et al*., 2008). Because of non specific disease symptoms, it cannot be excluded that this pathogen could be more widespread than known.

As consequence of the rapid spread of collapse of melon in Italy (Chilosi *et al*., 2008) and in Central Italy (Buzi *et al*., 2004), the constant monitoring of the disease as well as investigations on aetiology, epidemiology, prevention and control became necessary.

The present survey was performed in the period 2008-10 with the aim to:

- establish the occurrence of collapse throughout the melon-producing areas in cultivated in greenhouse in the seaside producing area in the North Latium and verify the identification of isolates collected according to morphological analisis;
- estimate the occurrence on root during the life cycle of melon from transplant to harvest;
- evaluate the on field resistance of genotypes cultivated in the area.

MATERIALS AND METHODS

Field surveys

Surveys of 100 melon farms throughout melon greenhouse production area (70 ha) in North Latium were conducted in 2008 - 2010. Farms ranged in size from 10

to 100 greenhouse (300 mq each). The farms were surveyed in Mars–June period, at the end of the cropping season, when plants approached maturity and symptoms of vine decline in the canopy appeared as patches of wilted or death plants.

Sampling and isolation of fungi

Plants showing collapse were sampled collecting all parts of the root tissue and examined for disease symptoms. Roots of each plant were exposed by carefully washing the soil away and for isolation, root fragments were surface sterilized for 1 min in a sodium hypochlorite solution (1,5% active chlorine) and washed twice with sterile water. Sixteen root segments per plant from affected areas of tissue were transferred onto Potato Dextrose Agar (PDA) containing 0.5 mg/ml streptomycin sulphate (PDAS). Plates were examined daily for fungal growth for 7 days, and hyphal tips from all colonies were transferred to PDA for subsequent growth and sporulation. Colonies of *M. cannonballus* were identified according to characteristic morphological features (Martyn and Miller, 1996).

Fungal pathogen incidence was evaluated as the percentage of pathogen isolated in the fields.

Evaluation of genotype resistance

The varieties of melon grown in the area have been classified in: early melon (EM), late melon (LM), melon rootstock (MR) and squash rootstock (SR).

Melon plants sampled from infected fields were evaluate for disease symptoms and rated on the following scale: 0 – no symptoms; 1 – no symptoms wilting, but root lesions; 2 – wilting at the end of harvest and root rot; 3 – wilting during the harvest and root rot; 4 – wilting before the harvest and root rot; 5 – death plant before the harvest.

Genotype resistance were estimated as disease severity (DS) by the following formula:

- $DS = (\Sigma \text{ all Rating})/(\text{Number of Observations x Highest Rating})x100.$

Statistical analysis

Data were subjected to analysis of variance and the means compared with Tukey's test (p=0,05) using GraphPad Prism software (San Diego, CA, USA).

RESULTS

On the basis of the phytopathological analyses, the main soil-borne diseases that affect melon in greenhouse production in the North Latium are reported in Table 3.1.

M. cannonballus was isolated in high percentage on selective grown medium from diseased melon roots in surveyed fields and the colonies were identified according to morphological features. No different soil-borne fungal pathogens involved in the vine decline complex, such as *A. cucurbitacearum*, *R. vagum* or *P. tabacinum* were isolated.

Table 3.1. Results of monitoring soil-borne melon diseases in fields during 2008-2010 in North Latium.

Diseases	Pathogens	Diseases occurrence		
		2008	2009	2010
Melon collapse	*Monosporascus cannonballus*	24%	30%	28%
Fusarium wilt	*Fusarium oxysporum* f. sp. *melonis*	25%	24%	27%
White mold	*Sclerotinia sclerotiorum*	6%	4%	10%
Damping-off	*Pythium* spp. *Rhizoctonia* spp.	5%	7%	4%

The most common and yield-limiting soil-borne disease of the coastal melon producing area of North Latium can be considered collapse and *Fusarium* wilt, since they determined yield losses up to 70%.

In years 2008 – 2010, *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *melonis*, race 1,2, has been monitored in the range between 24% to 27% of the surveyed farms; the attack usually occur on not-grafted susceptible varieties (Fig. 3.1 and

Fig. 3.2). The disease is favoured by cold climate during spring and water excess.

Collapse or sudden wilt, caused by *Monosporascus cannonballus*, was the most dangerous soil-borne disease in the melon grown area. Following first disease focus, by on-farm(few plants attacked per greenhouse), the pathogen can spread quickly, favored by monoculture and farming operations unfit for containment of inoculum, such as lack of sanitation practices.

The high greenhouse temperatures (above 30 °C), which occur in the environment before the end of the melon crop cycle, are conducive for early attacks, that result in qualitative damage (small fruits, low sugar content) and in complete yield losses in the most severe cases (Figure 3.3). The disease has been reported in the territory of Pescia Romana (VT) in 2002, while upon observation of the present survey, it was observed 28% of farm monitored damaged by *M. cannonballus* in spring 2010. The collapse is present only in greenhouse and generally takes place at the end of the growing season (late April), but during warm and dry spring, in coastal areas are possible attacks by the end of March.

The results of genotype resistance of *M. cannonballus* determined in each field are shown in Table 3.2.

Table 3.2. Resistance of *M. cannonballus* in melon varieties greenhouse grown in North Latium during 2008 – 2010. Data followed by the same letter on the columns are not statistically different at P≤0.05 by Tukey's test.

Melon varieties	Disease severity		
	2008	2009	2010
EM	3,6a	3,7a	3,4a
LM	2,8b	2,8b	2,6b
MR	4,1a	4,2a	3,9a
SR	0,1c	0,2c	0,1c

In years 2008- 2010, melon rootstocks (MR) and early melon (EM) are the most susceptible varieties to collapse, with high disease severity not different significantly between EM and MR. Commonest symptoms are wilting, lessoned and rooted roots, that result to death of plants before the harvest. Fruits are small, with

a low sugar content and therefore no marketable.

Late melon (LM) varieties are tolerant to collapse; disease severity is low and in this case, despite the presence of lesions and rots on the root apparatus, fruit production is valuable (Fig. 3.4).

Squash rootstocks (SR) are fully resistant to *M. cannonballus* (Fig. 3.5). Disease severity is very low (between 0,1 to 0,2 in three years) and rot symptoms on roots are likely produced by other soil-borne pathogens such as *Pythium* spp., *Rhizoctonia* spp., nematodes (Fig. 3.6).

Fig. 3.1 Attack of *Fusarium* wilt in greenhouse grown.

Fig. 3.2 Symptomatology of *F. oxysporum* f.sp. *melonis* in susceptible melon plant.

Fig. 3.3 In North Latium, melon collapse is the most dangerous soil-borne disease in greenhouse grown.

Fig. 3.4 Late melon is tolerant to *M. cannonballus* and disease severity is low.

Fig. 3.5 Squash rootstock is very resistant to *M. cannonballus*.

Fig. 3.6 Root-knot nematode (*Meloidogyne* spp.) induced galling of squash roots.

DISCUSSION

Results from the present work confirmed that protected melon in the coastal producing area of North Latium have been affected by an increasing number of destructive soil-borne fungal pathogens, which have become one of the yield-limiting factor to this crop. This might result from different changes in cultural practices during the last years, but the monoculture and improper soil environmental conditions appears the be the main factors as observed by Bruton *et al*. (2000) in USA.

Inadequate rotation increases severity of some soil-borne diseases due to soil sickness. Languasco *et al*. (2000) reported that just 2 years of melon monoculture are enough to reach high levels of soil-borne inoculum of *F. oxysporum* f.sp. *melonis*. It has been observed that fertiliser-irrigation, which represents the common irrigation system, reduce the root exploration, thus determining a reduced root functionality in the case of attack of soil-borne pathogens. (Krikun *et al*., 1982). Moreover, high fertilization regimes on melon monoculture was found to increase soil salinity, thus promoting some soil-borne diseases, such as *Macrophomina phaseolina* and *M. cannonballus* (Nischwitz *et al*., 2004; Cohen *et al*., 2007).

Collapse is caused by different of fungal species belonging to different genera, even

if one pathogen may predominate (Martyn and Miller, 1996; Pivonia *et al*., 1997; Aegerter *et al*., 2000; García Jiménez *et al*., 2000). It has been suggested that the fungal pathogens causing such diseases act similarly by suppressing the root system (Pivonia *et al*., 1997). In this study, a number of fungi were isolated from symptomatic roots of melon in the monitored production areas. The pathogen which is mainly associated with this disease is *M. cannonballus,* identified on the basis of morphological characteristics. The identification was confirmed by PCR methods with species-specific primers and DNA sequence data since 2008 (Chilosi *et al*., 2008). In this study, RFLP and sequence analysis showed the existence of a substantial homogeneity among Italian isolates of *M. cannonballus.* This result is consistent with previous observations which indicated that *M. cannonballus* population is characterised by a little variation based on morphological, pathogenetic and molecular studies (Martyn, 2002).

The frequency of isolation of fungal species varied with greenhouse locations. The predominance of a given species may be linked to different factors. On the basis of results obtained, the spread of the disease is related mainly on the cultivar or rootstock used. The commonest rootstocks used for preventing *Fusarium* wilt are very susceptible to collapse *M. cannonballus*. Therefore, it may be possible that the cultivation of this susceptible host has been opened the way to the spread of the disease.

Other reason, that may explain the rapid spread of collapse may be related to the earliness of the cultivated melon genotypes. The early melon cultivars are susceptible to collapse, causing fruits concentration and weak root system. This feature results in wilting before and during harvest, followed by death of plants. Conversely, late melon cultivars appear more tolerant to collapse due to the scalar maturation of fruits, even if climatic conditions favorable for *M. cannonballus* (high spring temperature) potentially cause severe attack before harvesting, as occurred during the outbreak of 2003 (Buzi *et al*., 2004). In this respect, it has to underline the general increase of average temperature during the last decade that might have favored the onset of the disease caused by this macro term pathogen as well as the

development of the infection and spread of the disease. Squash rootstocks appeared to be full resistant to the disease, and represent an effective tool for preventing the disease. However, grafting of squash results in quality with parameters often not acceptable.

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CHAPTER 4

Assessment of defense strategies against collapse of melon in Central Italy

Roberto Reda

Department of Plant Protection - Tuscia University – Viterbo - Italy

ABSTRACT

In present study a survey of conventional measures for preventing collapse by *Monosporascus cannonballus* in the coastal area of Central Italy was conducted with the aim to define that measures can be integrated in a sustainable prevention control. Metam sodium fumigation is effective in containing the development of disease, but its efficacy progressively declines after repeated applications. Soil solarization which is performed by most of farmers was confirmed to be ineffective in controlling the disease. Grafting on *Cucurbita* species is effective in controlling the disease whereas melon rootstock is fully susceptible to collapse. The present results indicate that the measures built up in melon producing area in Province of Viterbo are un-sustainable whereas are effective only few tools such as fumigation and grafting on squash. Therefore an integrated collapse management within a sustainable approach is strongly need.

Keywords: grafting, fumigation, melon rootstocks, *Monosporascus cannonballus*, squash

INTRODUCTION

Monosporascus root rot and vine decline caused by *Monosporascus cannonballus* is a destructive disease of melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) worldwide (Chilosi *et al*., 2008). The pathogen survives in soil as spherical and thick-walled ascospores, which germinate under the stimuli of root exudates and soil microflora (Stanghellini *et al.,* 2000). Perithecia contain numerous ascospores which are discharged into soil at the end of crop (Mertely *et al*., 1991; Stanghellini *et al*., 2000, 2004; Waugh *et al*., 2003). Control of the disease upon establishment in the soil is problematic due to durability and viability of the reproductive structures. Moreover, the pathogen is a thermophilous fungus, resistant to solarization. Integrated control measures of *Monosporascus* collapse has been reviewed by Cohen *et al* (2000). Data concerning collapse control from literature are reported in chapter 1. Cohen *et al.* (2000) have included in the integrated approach different measures, such resistance, grafting, effect of irrigation regime, improved soil solarization, fungicide application, soil fumigation alone or combined with soil solarization. The measures built up in these studies concern mainly open field crops in sites with different pedo-climatic condition. In the case of greenhouse crops it has to be considered to set up proper integrated tools capable not only to protect plants in already infected soils, but also to prevent the onset of the disease new melon cultivations and in greenhouse where the disease has not yet manifested. Moreover, the prevention of collapse melon has to include sustainable measures that can improve the physical, chemical and biological fertility of soil, which represents the prerequisite for a effective and sustainable soil-borne diseases prevention. A study of such measures will be reported in chapters 5 and 6.

The main objective of this work was a survey on conventional measures for melon collapse control in the area where the study was conducted, with the aim to define that measures that can be integrated in a sustainable prevention strategies.

MATERIALS AND METHODS

Field surveys

Surveys of 30 farms throughout melon greenhouse production area (70 ha) in North Latium were conducted in 2008 - 2010. Farms ranged in size from 10 to 100 greenhouse (300 mq each).

All fields had a known history of the melon collapse and were planted with susceptible early cultivars grafted with melon rootstock (GRM).

In each years, plants were transplanted between first and second week of February.

The greenhouse fields were surveyed in Mars–June period, at the end of the cropping season, when plants approached maturity and symptoms of vine decline in the canopy appeared as patches of wilted or death plants. A plant was considered dead when irreversible wilting was evident.

Defense strategies

Defense strategies of control melon collapse in the grown area included: metam sodium fumigation, solarization and grafting rootstock squash. Early cultivar grafted with melon rootstock (GRM) was used as control.

Preplant soil fumigation with metam-sodium (MS) (sodium *N*methyl dithiocarbamate) is used in this area only to control *M. cannonballus*.

MS was applied through drip fumigation method (450 liter ha^{-1} at a water volume of 400 m^3 ha^{-1}) in November each year (trees months after transplanting) in the beds covered with polyethylene sheets. Plastic films were left on the fumigated beds, at which time they were perforated for planting. The plastic mulch has been removed from the field after crop growth and production.

Soil solarization (SO) is non chemical technique used in this area to control of many soil-borne diseases and pest. Prior solarization, in each greenhouse soil is mechanically cleared of weeds and watered to field saturation. Thereafter, transparent polyethylene sheets 8 m wide and 150 mm thickness are extended,

usually carried out for approximately 60 d during the hot summer months (July and August) for increased soil temperatures to levels lethal to soil-borne plant pathogens.

The grafting of melons onto *Cucurbita* spp. rootstocks "P360" (GRS) is a technique commonly used in this area to control *M. cannonballus*. In each farm, GRS were transplanted with within-row spacing of 1,5 m and between rows 2,50 m.

Evaluation of defense strategies

In each greenhouse field, plants showing wilting and from which it was isolated *M. cannonballus* were rated on the following scale: 0 – no symptoms; 1 – no wilting symptoms, but root lesions; 2 – wilting at the end of harvest and root rot; 3 – wilting during harvest and root rot; 4 – wilting in the first harvest and root rot; 5 – death plant before the end of harvest.

Defense strategies were estimated as disease severity (DS) by the following formula:

- $DS = (\Sigma \text{ all Rating})/(\text{No of Observation x Highest Rating})x100.$

Moreover, efficacy of treatment (ET) were estimated by the following formula:

- ET (%) = (1 – Treated DS/ Untreated DS)x100.

Statistical analysis

Data were subjected analysis of variance and the means compared with Tukey's test (p=0,05) using GraphPad Prism software (San Diego, CA, USA).

RESULTS

Results from the observation carried out in the surveyed farms in the growing seasons 2008-2010 are reported in Tab. 4.1.

Table 4.1. Disease severity of *M. cannonballus* in greenhouse grown melon in North Latium during 2008 – 2010 after application of defense strategies against vine decline. Data followed by the same letter on the columns are not statistically different (P<0,05).

Defense strategies	Disease severity (DS)		
	2008	2009	2010
GRM (control)	4,1a	4,2a	3,9a
MS	0,75b	1,1b	1,2b
SO	3,7a	3,7a	3,8a
GRS	0,1b	0,2c	0,1c

Collapse symptoms were particular high when early cultivars were grafted on melon rootstocks (GMR) (Fig. 4.1), with DS 4,1 in 2008, 4,2 in 2009 and 3,9 in 2010. In this case, yellowing and death of the crown leaves and a gradual decline of the vine as the plant approaches maturity was observed. Root systems were heavily affected with numerous discrete lesions and lack most of the secondary and tertiary feeder roots. In each years, high plant mortality was recorded before the end of the harvests (Fig. 4.2).

The fumigation with metam sodium is a popular practice in the area surveyed for vine decline control. Results about the efficacy of fumigation on the collapse severity indicate that this practice is effective in containing the development of disease. MS treatments reduced the severity of the disease relative to untreated greenhouse (Fig. 4.3 and Fig. 4.4).

However, the disease severity was found to significantly increase, with 0,75 in 2008, 1,1 in 2009 and 1,2 in 2010.

Solarization was shown to be quite infective in controlling the disease (Fig. 4.5). Typical symptoms of wilting and melon collapse were observed in greenhouse

solarizated before the harvest, with DS 3,7 in 2008, 3,7 in 2009 and 3,8 in 2010.

Grafting *Cucurbita* rootstock (GRS) were the best control strategies of vine decline (Fig. 4.6), whit DS 0,1 in 2008, 0,2 in 2009 and 0,1 in 2010.

Data expressed as efficacy ET (Table 4.2) support the observation that MS and GRS are good conventional practices for controlling collapse of melon by *M. cannonballus.*

Table 4.2. Efficacy of control strategies against *M. cannonballus* in melon grown in North Latium during 2008 – 2010. Data followed by the same letter on the columns are not statistically different (P<0,05).

Defense strategies	Efficacy (%)		
	2008	**2009**	**2010**
MS	86,3b	79b	74,6b
SO	10,4c	10,5c	3,5c
GRS	97,3a	95,6a	97,8a

Present results indicate that grafting squash rootstock is the best *M. cannonballus* control, with efficacy in 2008 – 2010 more to 95%; fumigation is effective, but the present data report a decline of efficacy of this product along the time of usage (Fig. 4.7). Soil solarization is not efficacy.

Fig. 4.1 Early melon grafted with rootstock melon is very susceptible to *M. cannonballus*.

Fig. 4.2 High plant mortality before the end of the harvests caused by *M. cannonballus* in early melon grafted with rootstock melon.

Fig. 4.3 In greenhouse, metam sodium treatment is effective to control melon collapse.

Fig. 4.4 Attack of *M. cannonballus* in no-fumigated greenhouse.

Fig. 4.5 In greenhouse, soil solarization is ineffective to control melon collapse.

Fig. 4.6 Grafting squash rootstocks is the best control strategies of *M. cannonballus.*

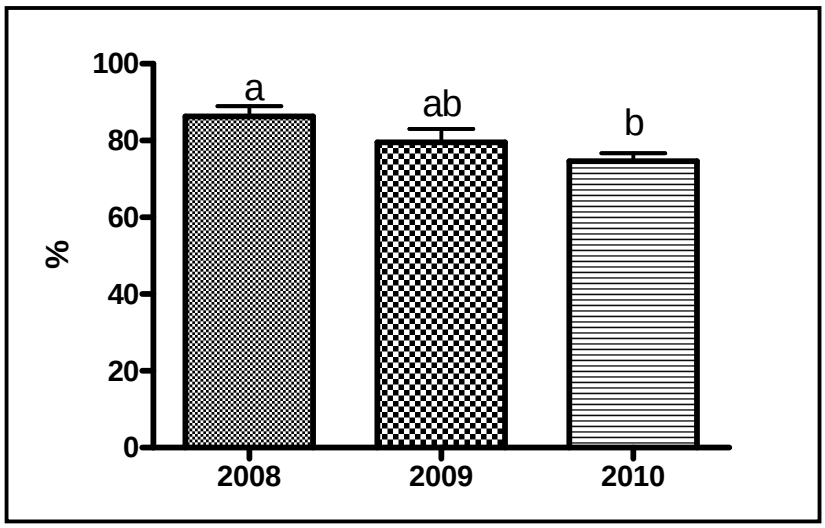

Fig. 4.7 Efficacy of MS fumigation during years 2008 – 2010. Means followed by the same letter are not significantly different at P≤0.05 by Tukey's test.

DISCUSSION

Control of collapse of melon by *M. cannonballus* is primarily by means of an integrated disease management strategies, involving the application of diverse control measures before and after planting (Edelstein *et al*., 1999; Batten *et al*., 2000; Cohen *et al*., 2000, 2005; Pivonia *et al*., 2002, 2010; Dias *et al*., 2004; Radewald *et al*., 2004; Aleandri *et al*, 2010; Reda *et al.,* 2010). Soil solarization is a nonchemical disease management technology, which improves plant growth and yield (Katan, 1981; Chen *et al*., 2000). Furthermore, soil solarization affects microbial activity in soil and results in increased antagonistic activity and induced inoculum soil suppressiveness (Gamliel and Katan, 1991). Collapse caused by the heat-tolerant soil-borne fungus *M. cannonballus* is scarcely controlled by current solarization technology applied to large soil volumes because the temperatures achieved are not high enough to kill ascospores (Cohen *et al*., 2000). Present results confirm this general observation that solarization alone are not sufficient in controlling collapse, whereas is effective against other important soil-borne diseases such as *Fusarium* wilt and other melon diseases (Trionfetti Nisini *et al*., 1999, 2000; Tamietti and Valentino, 2006). However, it has been shown that combining solarization with various fumigants at reduced dosage resulted in effective control of *M. cannonballus* and an increase in yield. The use of impermeable plastic films combined with solarization improved disease control and enabled further reduction of alternative fumigant dosages (Cohen *et al*., 2000; Pivonia *et al*., 2002).

In North Latium, drip fumigation with metam sodium is the standard for control of *M. cannonballus* because this method is easy to apply and the use of impermeable film enable low dosages of fumigant. Preplant MS fumigation of melon greenhouse was effective at reducing collapse following a single and two consecutive treatments, but after three applications there is reduction of effectiveness (Fig. 4.7).

This effect may be due to an increase of accelerated degradation of MS in soil after repeated application of the fumigants under field conditions, for to soil enrichment

of microbial populations capable of rapidly degrading this pesticide or its active degradation components (Di Primo *et al*., 2003). Warton *et al*. (2001) reported that enhanced biodegratation of MS is caused grampositive heat-resistant bacteria (resembling *Rhodococcus* and *Bacillus* spp.). The accelerated chemical degradation was also accompanied by a comparable reduction in pathogen control (Di Primo *et al*., 2003; Gamliel, 2008; Triky-Dotan *et al*., 2009). Moreover, it was reported that metam sodium significantly reduced the rate and extent of reproduction by killing pathogen hyphae in infected roots, but it had no effect on the viability of ascospores. Since ascospores are the primary source of inoculums in soil, metam sodium will not be effective as a preplant fumigant (Radewall *et al*., 2004).

Also fumigation, by producing a biological vacuum, avoids any kind of competition by beneficial soil micro-organisms and renders more quick and effective survived pathogen inoculum. More biological complexity and variability was found in soils collected outside greenhouse, in absence of the effect of chemical treatments (Antonelli *et al*., 2007).

Present results confirm that grafting on squash genotypes is highly effective mean for preventing the disease in soils infected by *M. cannonballus*. The grafting of muskmelons and watermelons onto *Cucurbita* sp. rootstocks is a technique commonly used to control these diseases in the Mediterranean region. The success that grafting melons onto *Cucurbita* rootstocks has in reducing the incidence of *M. cannonballus* 'vine decline' and increasing yield has been previously reported (Cohen *et al*., 2000,2005). In Spain muskmelon has been routinely grafted onto *C. maxima x C. moschata* to control *Fusarium* wilt (Miguel *et al*., 2004). This practice has also been used in Spain to control *Acremonium* collapse in watermelon crops (Armengol *et al*., 1998). Resistance/tolerance to collapse and reduced symptom development may be linked to the vigorous root systems of squash (Fita *et al*., 2007). However, it has been shown that *M. cannonballus* as well as *A. cucurbitacearum* are capable of infecting squash tissues (Alfaro-Fernández and García-Luis, 2009). Moreover grafting on *Cucurbita* species is often problematic in the production, since the quality of product is lower than that of un-grafted

varieties. Another risk is represented by outbreaks by soil-borne pathogens of cultivated squash, such as *Plectosporium tabacinum*, (Jimenez and Zitter, 2005).

Also, in case of continuous of mono-crop, may be occur forms of the pathogen virulent to *Cucurbita* rootstocks.

Indeed, melon rootstocks that are commonly used for preventing *Fusarium* wilt caused by *F. oxysporum* f. sp. *melonis* was shown to be fully susceptible to collapse by *M. cannonballus*. It is like that the cultivation of susceptible varieties such as melon rootstock have favoured the onset and the quick spread of the disease.

Taking together, the present results indicate that the measures built up in melon producing area in Province of Viterbo are un-sustainable and effective only by few tools such as fumigation and grafting on squash. Therefore, an integrated disease management oriented to fertility maintenance, which ensures crops rotation along with integrated control mesures, is strongly need to minimize inoculum of *M. cannonballus* in soil. Integrated and sustainable strategies represent an effective tool for limiting outbreaks of soil-borne pathogens, thus reducing losses and the need for synthetic chemical treatments.

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CHAPTER 5

Effect of compost amendment on melon collapse severity

Roberto Reda

Department of Plant Protection - Tuscia University – Viterbo - Italy

ABSTRACT

The fungal plant pathogens *Monosporascus cannonballus* is among the most damaging soil-borne plant pathogenic fungi of melon production under greenhouse conditions. Within a sustainable disease management strategy of horticultural crops the use of compost is particularly useful due to its general capacity to decrease or suppress soil-borne fungal diseases. The aim of this study is to isolate antagonistic fungi from compost made with green and municipal waste to evaluate *in vitro* their individually effects on *M. cannonballus*. Moreover, field experiments were carried out to determine the effects of composts on the incidence of these diseases. Antagonistic fungi were evaluated on the basis of their mechanism of antagonism *in vitro*. The antifungal activity was studied via the dual culture technique. Fungi were identified by morphological and molecular approach. Prevalent antagonistic fungal species were found *Trichoderma asperellum*, *Penicillium* spp. and *Aspergillus* spp. Dual culture experiments showed that all tested fungi significantly inhibited the mycelial growth of *M. cannonballus* comparatively to the untreated control. Experiments under greenhouse conditions have shown that compost amendment is capable to reduce the severity of collapse caused by *M. cannonballus* on melon.

The present results show that compost amendments can play an important role in

reducing economic losses from soil-borne diseases of melon, especially under greenhouse conditions.

Keywords: antagonistic fungi, *Aspergillus* spp.**,** compost, *Monosporascus cannonballus, Penicillium* spp., soil-borne diseases management, *Trichoderma asperellum*

INTRODUCTION

Soil-borne fungal and oomycete plant pathogens are considered among the major factors limiting the productivity of agro-ecosystems, since they are often difficult to control with conventional strategies such as the use of resistant genotypes and synthetic fungicides. Control of collapse by *Monosporascus cannonballus* is extremely problematic considering that ascospores can survive in soil for several years also in absence of the host and that the majority of melon cultivated genotypes are susceptible. The occurrence of collapse as well as other soil-borne diseases is correlated with the massive presence of inoculum in the soil and the presence of microorganisms that promote the infection (Stanghellini *et al.*, 2000), but also to the microbiological status of soil. Conventional farming practices are taking advantage of very effective production tools, but which inevitably lead to an imbalance in the microbiological telluric communities.

A distinct microbial community is established in a given environment as a consequence of the community evolution of individual component microorganisms (Fukui, 2003). According to the "Community Theory" (Caldwell *et al.*, 1997), microorganisms proliferate not as individual species but as a community in response to changes in the environment, evolving into a biological network that is most suitable for their survival and/or further growth. During this process, individual microorganisms interact and communicate with each other by sharing their limited genetic information in order to exploit available resources. Consequently, a functional community comprising many different species is formed. For soil microbial communities, amendment with organic matter can be

employed to apply selective environmental advantages in order to transfer microbial communities to newly evolved biological consortia with distinctive functions. In this respect, the use of compost as organic amendment can improve soils by contrasting the development of soil-borne pathogens. Many studies have reported that the incorporation of composts rendered soils suppressive to various soil-borne pathogens.

Very often, the cultivation of vegetables in open field and greenhouse runs without the addition of organic matter. This aspect leads to a gradual depletion of organic matter with serious implications from both nutritional and microbiological standpoints. For these reasons, the soil conservation in agriculture is central to a number of actions at both European and World level. Soil is essentially a non-renewable resource and a very dynamic system, which performs many functions and delivers services vital to human activities and ecosystems survival. The reduction of soil organic matter content is of worldwide concern (Directive (COM(2006) 232). Soil organic matter is essential for maintaining soil quality by improving the biological, physical and chemical soil conditions. This reduction has multiple causes, but increased mineralisation of organic matter due to intensive soil tillage and use of mineral fertilizers (leading to decreased application of organic matter) are among the most important. If current agricultural practices are maintained, an increase in soil organic matter is obtained only by external organic amendments. Among these, compost belongs to the most stable sources of organic matter. Compost and digestate from bio-waste are under-used materials. While offering an excellent contribution to EU resource efficiency and to the improvement of carbon-depleted soils, in many Member States demand suffers from a lack of end-user confidence, caused mainly by insufficient standardisation of compost composition and quality (COM(2010)235 final).

Soil organic matter (SOM) plays a major role in the carbon cycle of the soil. Indeed, soil is at the same time an emitter of greenhouse gases and also a major store of carbon containing 1,500 gigatons of organic and inorganic carbon. Around 45% of soils in Europe have a low or very low organic matter content (meaning 0-2%

organic carbon) and 45% have a medium content (meaning 2-6% organic carbon). The problem is of major importance in the Southern countries.

Though difficult to estimate, several studies demonstrate significant annual costs of soil degradation to society in the ranges of: erosion: €0.7 – 14.0 billion, organic matter decline: €3.4 – 5.6 billion, compaction: no estimate possible, salinisation: €158 – 321 million, landslides: up to €1.2 billion per event, contamination: €2.4 – 17.3 billion, sealing: no estimate possible, biodiversity decline: no estimate possible (SEC (2006) 1165).

In conventional agriculture the use of organic matter is often replaced by the use of synthetic fertilisers, which are very effective in plant productivity, but are also source of pollution and connected problems. Nutrients such as nitrogen and phosphorous are removed from soils by plant growth and need to be replaced. Nitrogen from mineral fertilizer is the major source of N input in EU countries although inputs from animal manure remain important, especially in regions of high livestock density. Excessive nitrogen surpluses, the difference between inputs and removals by crops, can pose a threat to the environment, leading to pollution of water, air and soil. The integration of environmental concerns into agricultural policies and practices aims to reduce current and potential pollution. The Water Framework Directive and the Nitrates Directive include direct and indirect measures to control the use of phosphorus in agriculture and to reduce phosphorus pollution from agricultural land to surface and ground waters. Although phosphorus is essential for the healthy development of people, animals and plants, it can accumulate in soils if more is applied to land in chemical fertilisers and manure than is removed through harvesting of crops.

The demand for phosphate is increasing, while global reserves are exhausted. The general estimate is that known resources will last for 100 years. There are no alternatives for phosphate as a key component of fertilisers, hence the implications for global food production are enormous and may eventually result in large-scale famine. Phosphorous shortage is expected to further complicate competing claims for food, energy and land and this could well lead to social-political turmoil. Better

defined and more effective composting and a wider range of recycling products can create alternatives for chemical fertiliser production and industry, assuming appropriate policy adaptations and effective organisational and value chains structures to deliver the benefits (http://phosphorus.global-connections.nl/phosphorus-depletion-final-report).

Many studies have reported that the incorporation of composts rendered soils suppressive to various soil-borne pathogens. Compost also can serve as a food base for endogenous microbes or introduced biocontrol agents to sustain suppression based on the activities of microbial communities (Hoitink, *et al.*, 1999). These results have been recently reviewed by Bonanomi *et al.* (2007). Soil enrichment with organic matter may be the most fundamental and sustainable approach to the biological control of soil-borne diseases. Various mechanisms are hypothesized to drive the phenomenon of disease suppression. Most of them are the result of interactions between antagonistic microorganisms and pathogens, via competition, antibiosis and hyperparasitism (Chen *et al.*, 1988; Lumsen *et al.*, 1983; Mandelbaun *et al.*, 1988, 1990; Bohem *et al.*, 1993; Alabouvette, 1999; Messiha *et al.*, 2007; Malandraki *et al.*, 2008). However, in certain studies disease suppression by compost also was found to result from resistance induced in the plant (Pharand *et al.*, 2002; Kavroulakis *et al.*, 2005; Yogev *et al.*, 2010).

Compost amendments of various sources have been described to possess a suppressive effect in melon against *Fusarium oxysporum* f. sp. *melonis* (Lumsden *et al.*, 1983; Ros *et al.*, 2005; Yogev *et al.*, 2006; Suárez-Estrella *et al.*, 2007) and *Pythium aphanidermatum* (Hadar and Mandelbaum, 1986). As far as we know, no information is available on the effect of compost amendments in limiting the occurrence of collapse of melon by *M. cannonballus*.

The aim of the present work was to evaluated:

- presence of antagonistic fungi in compost (isolation and identification);
- antimicrobial assays against *M. cannonballus*;

- the role of a compost amendment from urban waste in the containment of collapse by *M. cannonballus* by pathogenicity analysis in naturally infected soils.

MATERIALS AND METHODS

Compost characteristics

The compost from urban waste used in the present research was kindly supplied by AMA SpA (Azienda Municipale Ambiente, Roma – Italy). Chemical and physical characteristics were supplied by manufacturer (Table 5.1).

TABLE 5.1. Compost composition of the AMA establishment whit values indicated

PARAMETER	U. M.	Value	LIMIT (D. Lgs 217/2006)
pH		8	6-8,5
EC	µS/cm	4,1	
RH	%m/m	23	Max 50
N organic	% N tot	87	Min 80
C organic	% C SS	33	Min 25
C/N		16	Max 25
Cd	mg/Kg s.s.	<0,5	Max 1,5
Cr	mg/Kg s.s.	< 0,5	Max 0,5
Hg	mg/Kg s.s.	< 1,0	Max 1,5
Ni	mg/Kg s.s.	11	Max 100
Pb	mg/Kg s.s.	51	Max 140
Cu	mg/Kg s.s.	54	Max 230
Zn	mg/Kg s.s.	137	Max 500

Isolation of antagonistic fungi

Compost samples were then homogenized; then they were air-dried, sifted with 2 mm mesh sieves, and stored at 4 °C until processing. Determination of the adequate sample suspension, dilution and aliquots to be added to the culture medium was performed as described by Gil *et al.* (2009). To evaluate the fungal populations in compost, the dilution plate count technique was applied (Hirte, 1969). The culture media evaluated were potato dextrose agar (PDA,) (Oxoid,

Unipath Ltd, Basingstoke, England), and modified PDA (PDAm) through the addition of rose bengal (0,01 g l^{-1}), chloramphenicol (0,3 g l^{-1}), and streptomycin sulfate (0,01 g l^{-1}) after autoclaving the medium, pH 6.

The number of colony forming units per g of dry weight (CFU/g dwt) was calculated for each species or morphotype.

Identification antagonistic fungi

Morphological identification of fungi was made from cultures grown on PDA at about 21 °C for bout ten days following observation of colony character, growth rates in culture, conidia production, pigments, conidiophore branching and aggregation. For molecular identification, *Trichoderma* isolates were grown in potato dextrose broth (PDB) (Sigma) for 5 days at 25C°. Colonies were then harvested by vacuum filtration through cheesecloth were immediately frozen in liquid nitrogen. Frozen mycelia were ground into a fine powder in a mortar and pestle. DNA from mycelium was extracted by methods of Lee and Taylor (1990). Ribosomal ITS fragments were amplified with primers ITS1 and ITS4, beginning with 0,1-1.0 ng of total genomic DNA in an automated thermal cycler (C100, Biorad), as described as White *et al*. (1990).

Antimicrobial assays

The antagonist fungi were evaluated against *M. cannonballus* (isolated MCR) by dual culture technique as described by Morton and Strouble (1955). The method consists of placing an active mycelial disc (0,6 cm in diameter) of the pathogen, 1 cm from the edge of a 9 cm Petri plate containing freshly prepared PDA medium. Another disc (0,6 cm) of the antagonistic fungi was deposited in a diametrical opposed position 1 cm away from the other set of the plate. The experimental design used was a completely randomized with Petri dishes for each isolates. In control plates (without antagonist), a sterile agar disc was placed at opposite side of soil borne inoculates isolates plates. Inoculated plates were incubated at 25±1 °C until the end of the incubation period (7 days after inoculation). Two, 5, 7, 10

and 15 days after the incubation period, radial growth pathogen isolates was measured and percent inhibition of average radial growth was calculated in relation to growth the controls as follows:

- L=[(C-T)/C]x 100,

where L is inhibition of radial mycelial growth, C is radial growth measurement of pathogen in control, T is radial growth of the pathogen in the presence of antagonist (Edington *et al*., 1971).

Fields trials

The effect of the suppression on the root rot and vine decline of melon was evaluated in a two years field experiment in an unheated greenhouse. The greenhouse was located in the melon producing coastal area of North Latium (central Italy, Province of Viterbo) (42°23'09.31"N; 11°30'46.10"E) in soil naturally infected with *M. cannonballus* (Chilosi *et al*., 2008). Melon seeds cultivar Proteo (Syngenta Seeds) were transplanting in two years: in February 2009, with compost application immediately before transplanting, and in February 2010, with compost application three months before transplanting. The compost was applied by substituting for each transplanting site a volume of 6 liters of soil with a same volume of compost. Minimal inputs were made for bed preparation and maintenance in the greenhouse in order to minimize undesired movement of soil and redistribution of inoculum.

Standard local cultural practices were employed for insect control, fertilization, irrigation frequency, and weed management. Treatments were arranged in a randomized complete block design with three replications and ten plants for replication in each year.

At the end of each trial, the plants were harvested, graded for disease, and the pathogen was isolated as above. Symptoms on crown, primary and secondary roots were rated on the following scale: 0, healthy; 1, 25% root surface with lesions; 2, 50% root surface with lesions; 3, 75% root surface with lesions; 4, roots extensively lesioned; 5, roots badly decayed. The disease index was calculated

using a modification of the McKinney's formula (McKinney, 1923).

Statistical analysis

Data were subjected to analysis of variance using GraphPad Prism software (San Diego, CA, USA).

Comparison of means in antimicrobial assays between different antagonistic fungi isolated from compost were made by the Tukey test ($P \leq 0.05$).

Comparisons of means in greenhouse experiments between compost amended and no-amended plants were made by the Student T test ($P \leq 0.05$).

RESULTS

Isolation and identification of antagonistic fungi

The isolation of antagonistic fungi was performed on PDA and mPDA from AMA compost sample. The results are presented in Table 5.2.

Table 5.2. Fungal entities isolated from AMA compost and their load (CFU/g dwt).

Isolated	Antagonist fungi	UFC/g dwt
A1	*Aspergillus* sp.	$1{,}75 \cdot 10^3$
A2	*Aspergillus* sp.	$5 \cdot 10^3$
A3	*Aspergillus* sp.	$1{,}3 \cdot 10^3$
P1	*Penicillium* sp.	$1{,}4 \cdot 10^3$
P2	*Penicillium* sp.	$7 \cdot 10^2$
P3	*Penicillium* sp.	$3 \cdot 10^2$
T1	*Trichoderma asperellum*	$1{,}8 \cdot 10^2$
Others	-	$6{,}1 \cdot 10^2$

Aspergillus and *Penicillium* isolates were identified as genus by morphological characters. The *Trichoderma* sample isolated was identified by both morphological and molecular tools. Based on these, *Trichoderma* isolate was identified as *Trichoderma asperellum* (T1). Among species identified, 7 isolates, illustrating representative species associated to compost sample, were subjected to farther characterization.

Antagonistic activity *in vitro*

Results from the dual culture assay showed that the selected antagonistic microorganisms inhibited the mycelial growth of the target fungal pathogens, with varying efficiencies (Fig. 5.1).

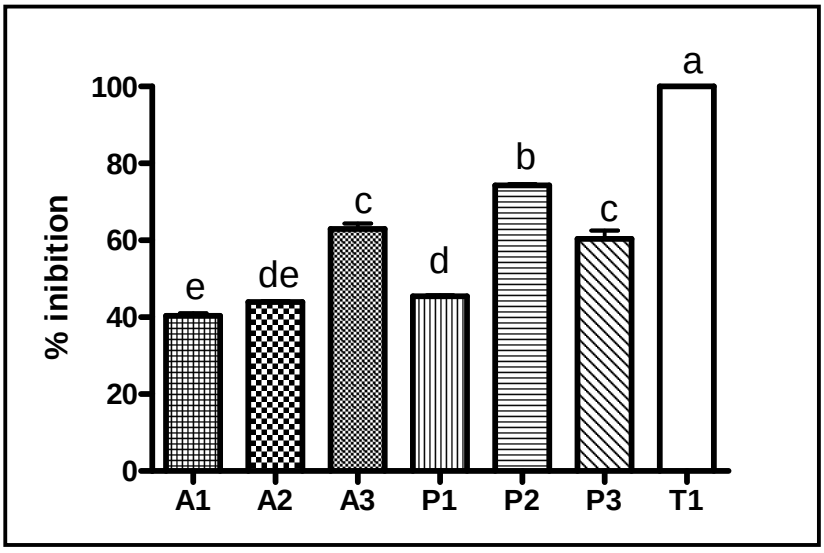

Fig. 5.1. Results from the dual culture assay between fungal antagonist isolated from AMA compost and MCR. Microorganisms inhibited the mycelial growth of the fungal pathogens with varying efficiencies. Means followed by the same letter are not significantly different at $P \leq 0.05$ by Tukey test.

Mycelial growth of *M. cannonballus* (MCR) completely covered the surface of the agar medium contained in control Petri plate within 15 d. By contrast, in this time interval *T. asperellum* (T1) reached the highest inhibition activity (100%) (Fig. 1.2b). The percentage of inhibitory by *Aspergillus* ssp. was 60,4% (A3) and 40,4% (A1) (Fig. 5.2c,d); moreover inhibitory activity by *Penicillium* ssp. was 74,3% (P2), 62,9% (P3) and 43,9% (P1) (Fig. 5.2e,f).

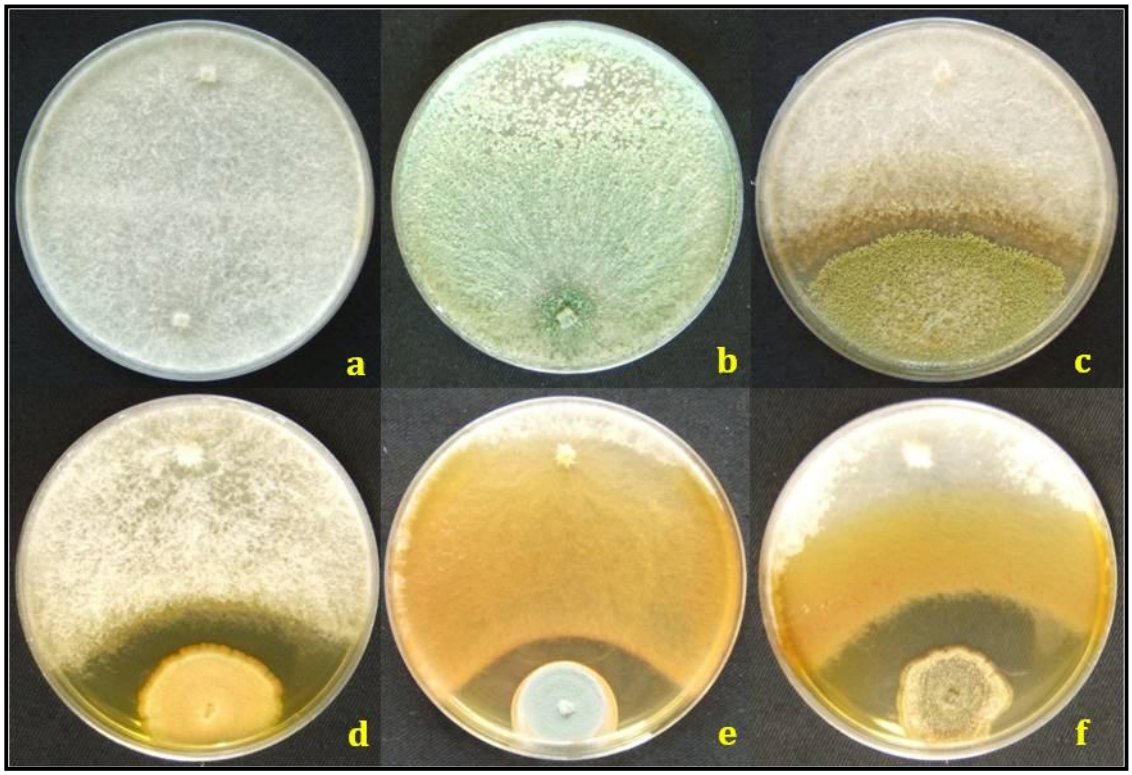

Fig. 5.2 *M. cannonballus* mycelial grown in dual culture. After 15 d control Petri plate were completed covered (a); *T. asperellum* inhibition activity was very good (b); *Aspergillus* spp. isolated had intermediate inhibition activity (c,d); *Penicillium* spp. inhibited mycelia grown with varying efficiencies (e,f).

Disease development in naturally infected soils.

Differences in the disease severity values of plants treated with compost and control plants were assessed at maturation stage in spring crop in plants grown in naturally infected soil under greenhouse conditions in both 2009 and 2010. Plants took an average of 80 d from transplanting to maturity. In both trials, the typical symptoms of the disease: rot of secondary and feeder roots, and reddish or corky lesions on the taproots were recorded when the control plants were removed from the soil. *M. cannonballus* was constantly re-isolated from samples of infected roots.

Plants grown on compost did not differ significantly from control plants in their disease and yield parameters in 2009 (Fig. 5.3), albeit average weight of fruits per plant from plants grown on compost amended soil was higher. The picture

emerged from 2010 was consistently different. The disease index of plants grown on compost was significantly lower than that of controls. Moreover, the average weight of fruits per plant was significantly higher (Fig. 5.4). Also, on the base of observation made on the whole root apparatus during plant grown, it was found generally a higher development of secondary roots on plants from amended soil compared of that of controls (Figure 5.5a,b). The end of the harvest, root of compost amended plant were less rot than those untreated control (Fig. 5.6a,b).

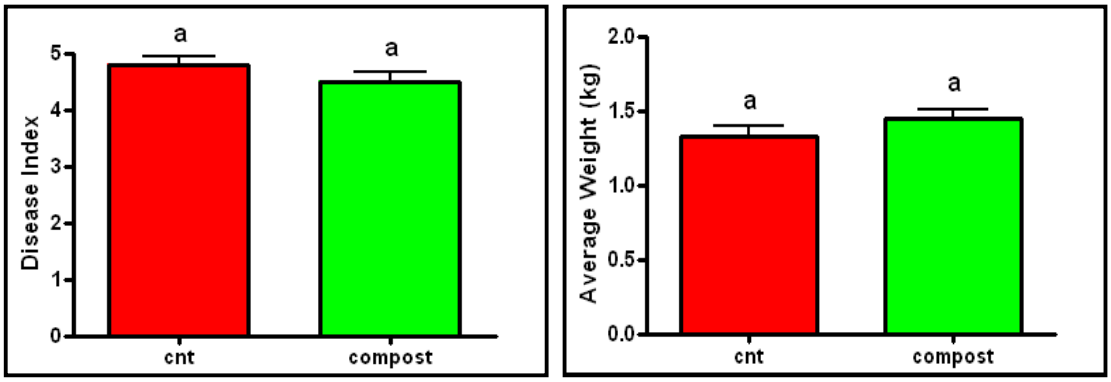

Fig. 5.3. In 2009 compost application was made immediately before transplanting. Disease index of roots and average weight of fruits is not significantly different between amended and control plant. Means followed by the same letter are not significantly different at P≤0.05 by Student's T test.

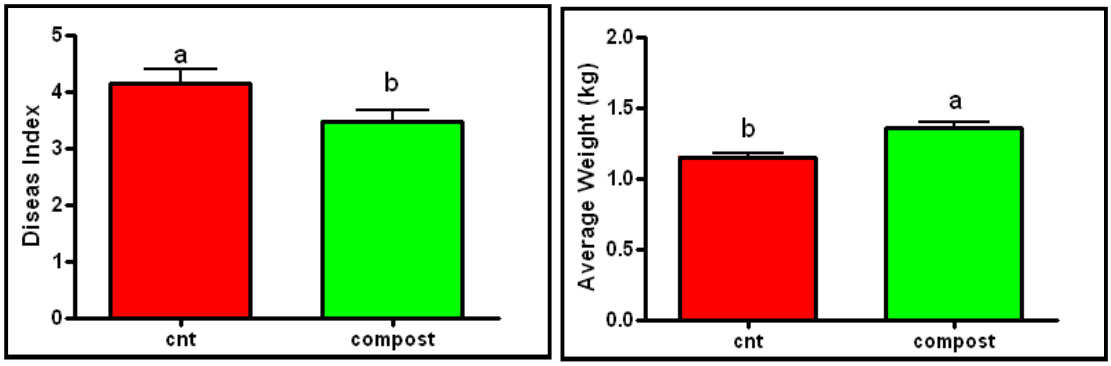

Fig. 5.4. In 2010 compost application was made three months before transplanting. Disease index of roots and average weight of fruits is significantly different between amended and control plant. Means followed by the same letter are not significantly different at P≤0.05 by Student's T test.

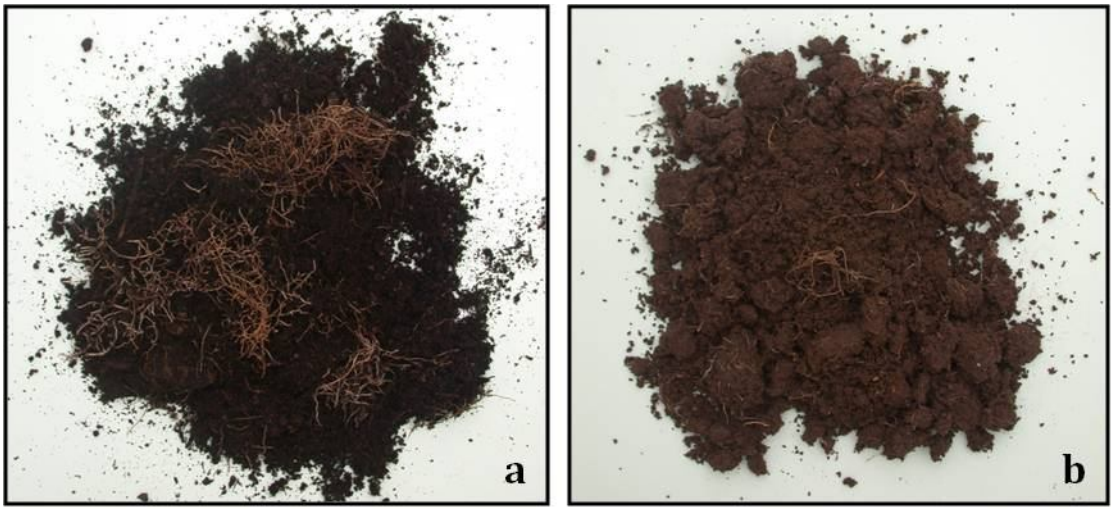

Fig. 5.5 Secondary root apparatus from plants grown in compost amended soil (a) and untreated control (b) in greenhouse naturally infected by *M. cannonballus*.

Fig. 5.6 Melon roots of compost amended plant (a) and untreated control (b) at the end of harvest in greenhouse naturally infected by *M. cannonballus*.

DISCUSSION

Different fungal isolate with antagonistic activity toward *M. cannonballus* have been isolated from compost from urban waste. The biological activity of this material is considerably high and its addition to the soil, therefore, may improve the soil biological activity by enriching the microbial population including that with an antagonistic activity against soil-borne pathogens. The antagonistic fungi isolated appear to possess diverse mechanisms of action. Combination of showing a differences and synergism in antibiotics as wells extracellular enzymes composition and activity is a promising approach to improve the control of plant diseases.

In the current study most of the antagonistic fungi selected were *Aspergillus* and *Penicillium* spp. They were shown to inhibit pathogen grown, thus indicating the production of antibiotics. *Aspergillus* and *Penicillium* isolates are the predominant fungal antagonists of *Sclerotium rolfsii* (Danon *et al*., 2010; Hadar and Gorodecki, 1991). Moreover, antagonistic *Aspergillus* spp. from compost amendments were found to be the main antagonistic population against *F. oxysporum* f.sp. *melonis* and *Fusarium* spp. attacking potato (Daami-Remadi, *et al*., 2006; Suàrez-Estrella *et al*., 2007).

Several different *Trichoderma* species were from compost with a suppressive activity (Pugliese *et al*., 2008). *Trichoderma hamatum* 382 has been identified as effective biocontrol agents in compost-amended substrates (Kwok *et al*., 1987; Hoitink, 2004). *Trichoderma* species appear to be interesting as potential biocontrol agents against *M. cannonballus*, as previously observed by the use of *T. virens* by Zhang *et al*. (1999).

In the present research, *T. asperellum* was shown to have the highest antagonistic activity among the tested fungal isolates. Thus, this *Trichoderma* species was confirmed to be effective biocontrol agent against many soil-borne plant pathogenic fungi, including, antagonising through antibiosis, nutrient competition and cell wall-degrading enzymes and by inducing resistance in plants (Liu *et al*., 2010). *T. asperellum* appear to be an important component of the compost

antagonistic population as previously found by Cotxarrera *et al*. (2002). This *T. asperellum* isolate named T34 was found to be a biocontrol agent effective against diseases caused by soil-borne pathogens such as *Fusarium oxysporum* (Cotxarrera *et al*., 2002) and *Rhizoctonia solani* (Trillas *et al*. 2006). In cucumber plants, T34 is also able to induce systemic resistance (ISR) against *P. syringae* pv. *lachrymans* (Segarra *et al*., 2007). This *T. asperellum* ISR is associated with increased peroxidase and chitinase activity in local and systemic cucumber tissues (Yedidia *et al*., 2003; Segarra *et al*., 2007). Moreover, application of high inoculum densities of T34 spores at the roots resulted in a systemic increase in levels of the signalling molecules salicylic acid and jasmonates (Segarra *et al*., 2007).

The findings resulted from the present research indicate that the use of compost as a soil amendment in melon under greenhouse provides a promising tool to be integrated in the sustainable management of soil-borne diseases. It has been shown in a previous research developed in order to test the same compost batch for growing melon in potting mixes peat-free that compost has a positive effect in plant growth and soppressiveness of *Fusarium oxysporum* f.sp. *melonis* (Parisella, 2010). The present data indicate that the compound has also a suppressive capacity against *M cannonballus* in on-field trials. Suppressiveness was found to be significantly higher than that of the untreated control in the 2010, but not in 2009. This can explained by the fact that in 2009 soil amendment with compost has been done before melon transplant at the end of January. In this period, the low temperature regime does not allow a proper activity of compost beneficial microorganisms as well as mineralization of organic matter, thus resulting in a scarce suppressive activity against the pathogen. Conversely, the soil amendment treatment was run three month before transplanting and might have favoured the disease containment due to the better environmental condition for beneficial microorganisms. Lumsden *et al*. (1983) reported that the suppressive activity of compost toward *Rhizoctonia solani* is associated with the longer is the time of soil incorporation into soil. The suppression disease of *M. cannonballus* may be due to activity and total microbial biomass generated from the compost degradation.

Thus, it is likely that increase suppressiveness activity is determined by the amount of microbial biomass antagonistic of plant pathogens (Chen *et al.* 1988).

The increase in yield might have been as consequence of the prolonged synergistic antagonistic activity of beneficial microorganisms and the a faster mineralization rate of organic matter.

We conclude that compost amendments can play an important role in reducing economic losses from diseases to melon growers, especially in greenhouse production where plants are more exposed to pathogens due to monoculture and higher agronomical inputs used.

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CHAPTER 6

Influence of the endomycorrhizal fungus *Glomus intraradices* on the development of collapse of melon by *Monosporascus cannonballus*.

Roberto Reda

Department of Plant Protection - Tuscia University – Viterbo - Italy

ABSTRACT

In the present research was analysed the effects of melon roots mycorrization with the arbuscural mycorrhiza *Glomus intraradices* on the development of *M. Cannonballus*, in soil naturally infected with the pathogen, as well as on the yield parameters of the melon plants. A preliminary experiment was conducted in laboratory to evaluate the level and the time of mycorrhizal colonization of the melon roots, inoculated with *Glomus intraradices*. The total shoot biomass in mycorrhized plants was significantly higher compared to the control. Effectiveness appeared to depends also to plant genotype. Conversely, roots development of mycorrhized plants did not differ from the control. In field trials, differences in the disease severity of plants inoculated with *G. intraradices* were assessed at the maturation stage in spring and summer crop in plants grown in naturally infected soil under greenhouse conditions. Inoculated plants significantly differed from control plants in their disease parameters in spring crop. The average fruit weight was also significantly higher in mycorrhized plants compared to the control. In the case of summer crop, mycorrhization with *G. intraradices* was associated nor with the containment of the disease, neither with an increase of yield parameters. Although promising, inoculation with AMF alone is not sufficient for the complete

prevention melon collapse, but it has to be integrated in a sustainable control strategy promoting fertility maintenance and restoration.

Keywords: *Glomus intraradices*, melon, *Monosporascus cannonballus*, disease containment, crop yield

INTRODUCTION

Microbial activity in the rhizosphere is a major factor affecting the availability of nutrients to plants and has a significant influence on plant health and productivity (Jeffries *et al.*, 2003). Soil–plant–microbe interactions are complex and there are many ways in which the outcomes can influence plant health and productivity (Kennedy, 1998). The results may be harmful, neutral or beneficial to the plants. A negative interaction is represented by the colonisation of melon rhizosphere and roots by *Monosporascus cannonballus*, the causative agent of collapse. This destructive disease occurs worldwide and it is characterised by an heavily attack of roots, which leads to plant wilt just before harvesting (Chilosi *et al*., 2008). Many factors affecting the onset of the disease are involved, including a modification of the quantitative and qualitative component of the microbial community (Stanghellini *et al*., 2000; Antonelli *et al*., 2007).

There are several groups of beneficial rhizosphere microorganisms. Some engage in well-developed symbiotic interactions in which particular organs are formed, such as mycorrhizas and root nodules, whilst others develop from fairly loose associations with the root (Jeffries *et al.*, 2003). In particular, among symbiotic micro-organisms, arbuscular mycorrhizal fungi (AMF), have considerable significance in the maintenance of soil health, fertility and prevention of plant diseases (Azcón-Aguilar and Barea, 1996; Barrea *et al.*, 2002). AM fungi, which belong to the order *Glomales* of the Zygomycetes (Rosendahl *et al*., 1994), biotrophically colonise the root cortex and develop an extramatrical mycelium which helps the plant acquire mineral nutrients and water from the soil. AM symbioses play a key role in nutrient cycling in ecosystems (Jeffries and Barea,

1994) and the external mycorrhizal mycelium, in association with other soil organisms, forms water-stable aggregates necessary for good soil quality; moreover AM fungi produce copious amounts of glomalin to create an environment for growth of their host plants (Wright and Upadhyaya, 1998). The increased capacity for nutrient acquisition resulting from mycorrhiza association could help the resulting stronger plants to resist stress. However, AM symbioses can prevent soil-borne plant diseases by inducing resistance trough the jasmonates mediated metabolic pathway (Pozo and Azcón-Aguilar, 2007; Kempel *et al.*, 2010). Consistent reduction of disease symptoms has been described for fungal and oomycete pathogens such as *Phytophthora, Gaeumannomyces, Fusarium, Chalara (Thielaviopsis), Pythium, Rhizoctonia, Sclerotium, Verticillium, Aphanomyces,* and for nematodes such as *Rotylenchus, Pratylenchus* and *Meloidogyne* (Azcón-Aguilar and Barea, 1996). AM-induced increase in resistance or a decrease in susceptibility requires the pre-establishment of AM and extensive development of the symbiosis before pathogen attack. Furthermore, the potential effectiveness depends on the virulence and inoculum potential of the pathogen present in the soil, since a high pathogen inoculum density in the rhizosphere may render ineffective any form of biocontrol, including that mediated by an AM symbiosis (Azcón-Aguilar and Barea, 1996).

Soil in the greenhouses where melon is grown is usually subjected to chemical treatments and mineral fertilisation including phosphorous that are detrimental to the AMF establishment (Bendavid-Val *et al.*, 1997; Schreiner *et al.*, 2001; Smith *et al.*, 2003). In this context, the inoculation of melon with AMF may represent an additional tool for preventing collapse by *M. cannonballus* within a sustainable approach.

The aim of the present research was to test:

- a protocol of mycorrhization, using a commercial AMF *inoculum* for colonization of melon roots;
- the effects of melon roots mycorrhization with the arbuscural mycorrhiza *Glomus intraradices* on the development of *M. cannonballus* in soil naturally

infected with the pathogen as well as on the yield parameters, in order to provide a basis for assessing the significance of a single AMF inoculation in the prevention of this disease.

MATERIAL AND METHODS

Plant growth and AMF inoculation

Fifty seeds of melon (*Cucumis melo*) *cv* Proteo (Syngeta Seeds) and fifty seeds of *cv* Dinero (Syngenta Seeds) were surface-sterilized in 1% sodium hypochlorite solution for 5 min, rinsed with sterile water three times and germinated in vermiculite at 26 °C for 48 h. After germination, plants were grown in a mixture of peat, by adding 4 g/l of $CaCO_3$, silicate sand and vermiculite (2 : 1 : 1, by volume), in separated small pots (Ø 6,5 cm).

Thirty plants of both cultivar were inoculated with AM, adding to the mixture 15 g/l of *Glomus intraradices* commercial AMF inoculum (Aegis, Italpollina); ten plants of both cultivar were used as control.

Pots were placed in a growth chamber with a timer set at 16/8 h light/dark photoperiod, at 21 °C. No additional fertilizer was added during the 2 months of plants growth.

Evaluation of mycorrhization

Five plants were controlled at 30, 45 and 60 days after inoculation (dai) to assess the mycorrhization. The root system, free of soil, was washed several times with water, stained with 5% ink-vinegar solution with white household vinegar and destained by rinsing in tap water (acidified with a few drops of vinegar) (Vierheilig *et al*., 1998).

Thirty root fragments for plant were mounted on two slides with a drop of glycerol and were observed under the microscope.

Using method of Trouvelot *et al.* (1986), it was estimated the level of mycorrhizal colonization of the root system, of each root fragment, and the abundance of arbuscules. Parameters of mycorrhization were calculated with MYCOCALC

software, available at http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html.

Evaluation of mycorrhization was expressed as:

- frequency of mycorrhiza in the root system (F%);
- intensity of the mycorrhizal colonization in the root system (M%);
- arbuscule abundance in the root system (A%);
- intensity of the mycorrhizal colonization in the root fragments (m%);
- arbuscule abundance in mycorrhizal parts of root fragments (a%).

Field trial

The effect of mycorrhization by *G. intraradices* on the root rot and vine decline of melon was evaluated in a two-season field experiment in an unheated greenhouse. The greenhouse was located in the melon producing coastal area of North Latium (Central Italy, Province of Viterbo) (42°23'09.31"N; 11°30'46.10"E) in soil naturally infected with *M. cannonballus* (Chilosi *et al.*, 2008). Melon plants (*Cucumis melo*) cv Proteo and cv Dinero (Syngenta Seeds) were inoculated with the *G. intraradices* as described above. Minimal inputs were made for bed preparation and maintenance in the greenhouse in order to minimise undesired movement of soil and redistribution of pathogen inoculum. Transplanting was done in February (spring crop) and June (summer crop). Standard local cultural practices were employed for insect control, irrigation frequency, and weed management. Chemical fertilization was carried out with products that did not contain phosphorus. Treatments were arranged in a randomised complete block design with three replications and ten plants per replication in each crop season. At the end of each trial, the plants were harvested, graded for disease, and the pathogen was isolated from symptomatic roots.

Statistical analysis

Data were subjected analysis of variance and the means compared with Student's T test (p=0,05) using GraphPad Prism software (San Diego, CA, USA).

Comparison of means of growth parameters between Proteo and Dinero were made by the Tukey test ($P \leq 0.05$).

Comparison of means of mycorrhizal parameters between Proteo and Dinero were made by the Student T test ($P \leq 0.05$).

Comparisons of means in greenhouse experiments between mycorrhized and no-mycorrhized plants were made by the Student T test ($P \leq 0.05$).

RESULTS

Plant growth

The total shoot biomass in terms of shoot height induced in melon plant by *G. intraradices* was significantly higher in inoculated plants compared to the control (Table 6.1). Inoculation appeared to be more effective on cv Dinero than on cv Proteo. Inoculation resulted in a constant significantly increase in shoot biomass from 30 to 60 dai after sowing on both plant genotypes (Fig. 6.1). Conversely, the inoculation was reflected in a less developed root system, which did not differ from the control.

Table 6.1. Growth parameters of 30, 45 and 60 dai melon plants mycorrhized with *Glomus intraradices*. Data followed by the same letter on the columns are not significantly different at P≤0.05 by Tukey test.

Cultivars	30 dai		45 dai		60 dai	
	Plant height (cm)	Roots length (cm)	Plant height (cm)	Roots length (cm)	Plant height (cm)	Roots length (cm)
Proteo control	13,88b	18,5a	20,58b	22a	28,37b	25,75a
Dinero control	16,74b	20,7a	21,33b	19a	24,5b	20,75a
Proteo mycorrhized	20,3a	22,62a	35,8a	17,7a	46,9a	23,1a
Dinero mycorrhized	20,59a	19,05a	33,8a	19,9a	41,7a	20,4a

Development of arbuscular mycorrhizal symbiosis

Determination of mycorrhization rates using the method of Trouvelot *et al.* (1986) demonstrated nearly complete colonization of the roots 6 weeks after inoculation (Fig. 6.2). Results of the level and the time of mycorrhizal colonization of the melon

roots are reported in Fig. 6.3. Not significant differences resulted in the mycorrhization parameters between cv Proteo and cv Dinero within 30 and 45 dai. At 60 dai, cv Dinero appeared characterised by significantly higher mycorrhization values than cv Proteo.

Fig. 6.1 Morphological features of 60 dai melon plants mycorrhized with *Glomus intraradices*. Dinero uninoculated control (a) and mycorrhized plant (b); Proteo uninoculated control (c) and mycorrhized plant (d).

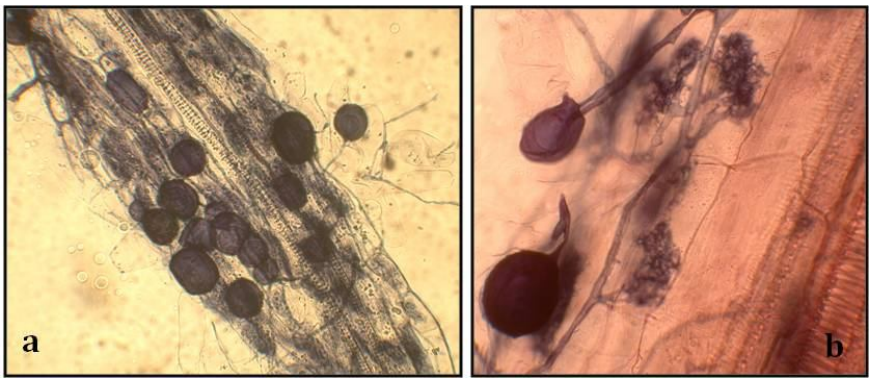

Fig. 6.2 Morphological features (400x) of *Glomus intraradices* on melon roots (a). Arbuscules and some intraradical vesicles clearly distinguishable (1000x) (b).

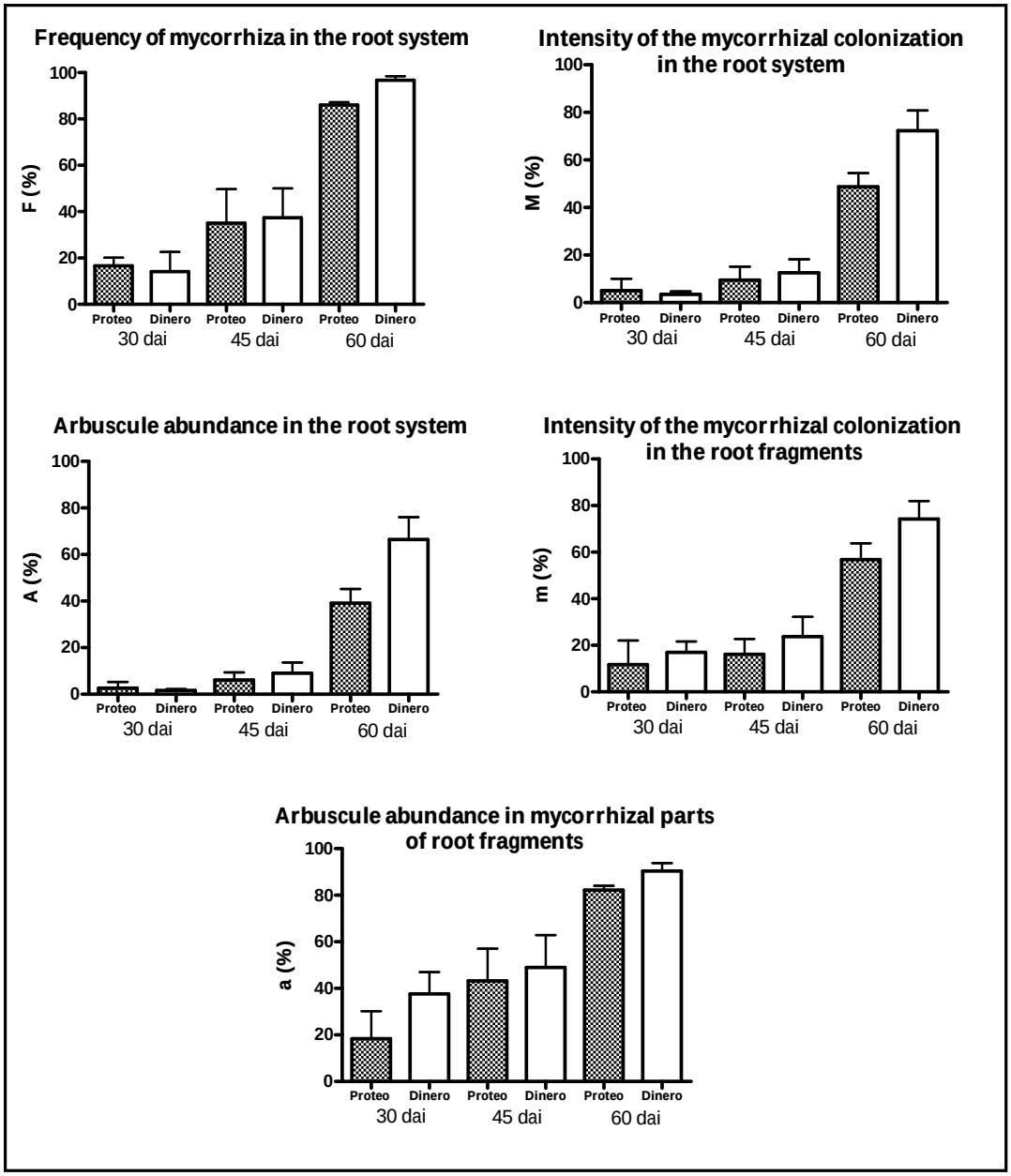

Fig. 6.3 Results of mycorrhization parameters at 30, 45 and 60 days after inoculation (dai) with *G. intraradices*. At 60 dai Dinero appeared characterised by significantly higher mycorrhization values than Proteo. Bars indicate standard error of the mean.

Effect of mycorrhization in collapse containement and yield in greenhouse disease development in naturally infected soils.

Differences in the disease severity of plants inoculated with *G. intraradices* were assessed at the maturation stage in both spring and summer crop in plants grown in naturally infected soil under greenhouse conditions (Fig. 6.4). Plants took an average of 60 days from transplanting to maturity, in summer, and 80 days in spring. In both summer and spring trials, the typical symptoms of the disease, rot of secondary and feeder roots, and reddish or corky lesions on the taproots, were recorded when the control plants were removed from the soil. *M. cannonballus* was constantly re-isolated from samples of infected roots.

Inoculated plants significantly differed from control plants in their disease parameters in spring crop. The average fruit weight was also significantly higher in mycorrhized plants than in that of control (Fig. 6.5). Conversely, the average yield per plant was not significantly enhanced in inoculated plants. In the case of summer crop mycorrhization with *G. intraradices* was associated nor with the containment of the disease neither with an increase of yield parameters (Fig. 6.6).

Fig. 6.4 Melon plants cultivated under greenhouse in naturally infected soil showing collapse symptoms 80 days after transplanting in spring crop. Plant uninoculated control (a) and plant mycorrhized with *Glomus intraradices* (b).

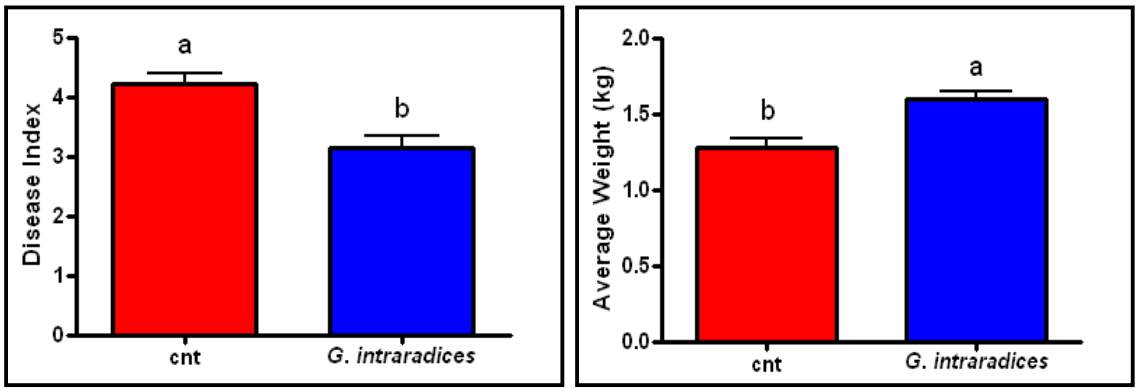

Fig. 6.5 In spring crop, disease index on mychorrhized roots and average weight of fruits are significantly different than control plant. Means followed by the same letter are not significantly different at P≤0.05 by Student's T test.

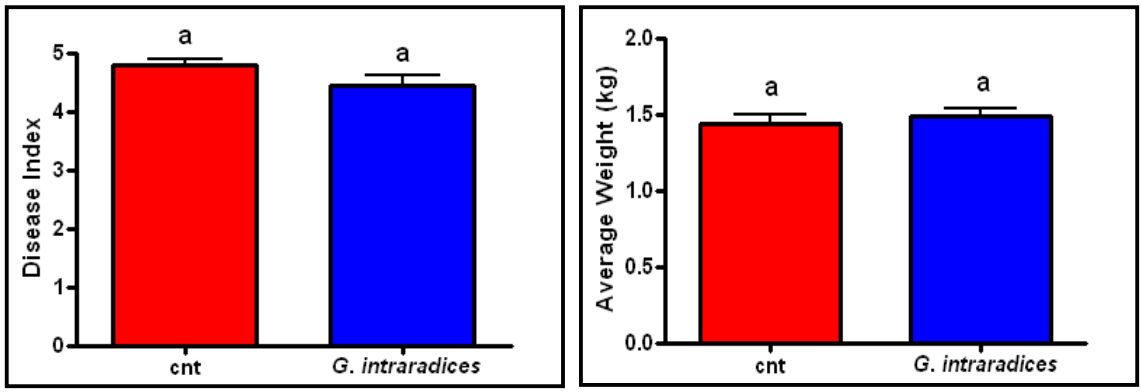

Fig. 6.6 In summer crop, disease index on mychorrhized roots and average weight of fruits not are significantly different than control plant. Means followed by the same letter are not significantly different at P≤0.05 by Student's T test.

DISCUSSION

Arbuscular mycorrhizal fungi develop intensively inside melon roots and within the soil by forming an extensive extraradical network and this helps plants considerably in exploiting mineral nutrients and water from the soil (Jeffries *et al.*, 2003). Phosphorus is the key element obtained by plants through the symbiosis and the evidence to support this is extensive (Smith and Read 1997; Koide and

Kabir, 2000). In exchange, mycorrhizal plants provide the fungus with photosynthetic C, which in turn is delivered to the soil via fungal hyphae. The extraradical hyphae of AMF therefore act as a direct conduit for host C into the soil and contribute directly to its C pools, bypassing the decomposition process. As a consequence of this, the amount and activity of other soil biota are stimulated (Jeffries *et al.*, 2003); however, this seems to be a selective phenomenon, since it stimulates in particular the microbes having antagonistic activity against soil-borne pathogens (Linderman, 2000). The reason for this phenomenon is unknown, but this observation clearly indicates that AMF could be useful biological tools for maintaining healthy soil systems (Jeffries *et al.*, 2003). In the light of the increasing cost of chemical inputs and a recalcitrant pathogen such as *M. cannonballus* resistant to agronomical practices, including solarization, inoculation of AM fungi may provide a more sustainable and environmentally acceptable alternative to these current practices. Therefore, the aim of the research was conducted to assess the possibility to reduce the damage of collapse by melon inoculation with a commercial isolate of *G. intraradices*. This isolate has been previously investigated as potential microbiological agent for the containement of melon fusarium wilt by *Fusarium oxysporum* f.sp. *melonis* race 1,2. It was found that mycorrhized melon plants are more resistant to the pathogen. It was observed also that the bioprotection conferred by this AM might be associated with the induction of resistance, since a consistent accumulation of IR markers, peroxidase and chitinase occurred in inoculated roots (Tardani, 2009). The present research evaluated firstly the mycorrhization parameters on melon during plant growth. An acceptable mycorrhization rate was established from the 30 days onwards, attaining maximum MYCOCALC parameters at 60 days in the range observed. It means that interaction begins during early plant growth. After transplanting in naturally infected soils, plants are challenged by pathogens by penetration and subsequent colonisation of parenchyma and vessels (Waugh *et al.*, 2005). Mycorrhization is associated with the enhancement of plant resistance by diverse mechanisms (Azcòn-Aguillar and Barea, 1996). However, a complete protection

was not achieve, probably because the heavy *M. cannonballus* inoculum resulted in a effective built up of the disease. A significant decrease of the disease index was observed on mycorrhized plant in the spring crop and this was reflected by a significantly superior average fruit weight compared to control. The results obtained in summer crop support our prediction that high soil temperatures would reduce AM fungal colonization (Martin and Stutz, 2004). In a study focused on effect of *M. cannonballus* infection and fruit load on water balance in melon, it was observed that plant water uptake started to decrease shortly before plant wilting and death (Pivonia *et al.*, 2002). Authors observed an extensive rise in tylose formation in xylem vessels and most vessels were plugged. Mycorrhization might have influenced positively the water uptake determining a more correct fruit load despite the presence of the disease. This effect might also affected the plant metabolism by inducing an earlier fruit maturation, even if the total plant yield was not affected.

The present results indicate that AMF are a promising opportunity to prevent the onset of this problematic soil-borne disease. The development of *M. cannonballus* collapse depends by different factors including the inoculum amount and composition of resident beneficial microbiological community in soil and rhizosphere. It is obvious that inoculation with AMF alone is not sufficient for the complete prevention of the disease. Although it is difficult to reach practical conclusions because of the complexity of the microbe-soil-plant system and the decisive influence of prevailing environmental conditions, it may nevertheless be possible to find the right combination of factors to exploit the prophylactic ability of AM fungi (Azcòn-Aguilar and Barea, 1996). Appropriate AM fungi must be used, preferably in association with other pathogen-antagonistic members of soil microbiota along with appropriate integrated cultural practices such as conservative systems and the use of compost.

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General discussion

Melon represents a strategic crop in the Italian agriculture. Production is typified in the spring and summer seasons. Winter crop is not possible to obtain due to the irradiation and pollination limits. In addition, winter imports from countries of south hemisphere is difficult due to the perishable characteristics of the product. Therefore, greenhouse early production of melon assumes a great commercial value. In the coastal area of North Latium, farmers produce an early melon with an excellent quality, which is sold at mid-May when the earlier Sicilian product is going to finish. It represents, therefore, a key product in the local economy. This importance, however, results in a highly specialized cultivation, obtained with a range of agronomic tools often inappropriate.

First of all in greenhouse grown, melon has being cultivated in monoculture for more than 10 years, resulting in most cases with problems of soil sickness. Soils were rarely treated with organic matter, but with heavy application of chemical fertilizers. Moreover, the irrigation regime located and continuously maintained on the same transplant site has resulted in the increase of salinity. Such conditions along with the general susceptibility of melon genotypes, led to an environment particularly conducive for the establishment of a number of soil-borne diseases, including root rot by *Rhizoctonia solani* (Corazza *et al*., 1992), white mould by *Sclerotinia sclerotiorum* (Chilosi *et al*., 2004) and fusarium wilt by *F. oxysporum* f. sp. *melonis* race 1,2 (Chilosi *et al*., 2008). The control management systems that have been introduced for limiting such diseases were fumigation, solarization and grafting on melon genotypes resistant to *Fusarium* wilt. Unfortunately, at reported in Chapter 3, genotypes resistant to *Fusarium* as well common early cultivars were completely susceptible to *M. cannonballus*, while solarization was ineffective. The fumigation gave initially good results, but later its efficacy was found to decline

(Chapter 4). Moreover, the rapidity and severity of collapse can generally associated with high temperatures level, which occurred in the last decade.

Results reported in chapter 3 indicate that collapse or sudden wilt of melon, caused by *M. cannonballus*, is the most dangerous soil-borne disease in the coastal area of North Latium.

Moreover, in chapter 4 our results indicate that collapse of melon is a disease hard to face. Soil solarization and grafting with melon rootstock are ineffective against *M. cannonballus*. Preplant metam sodium fumigation is effective at reducing sudden wilt; however, a decline in efficacy of fumigation was found if it is repeated over time. Only grafting on squash genotypes is a highly effective mean for preventing melon collapse, but repeated use may predispose the selection of virulent pathogens of *Cucurbita* rootstocks.

Therefore, an effective defense strategy can be obtained by integrating several sustainable control methods.

The two main strategies that can be introduced are:

1. to manage an integrated prevention system in new greenhouse cultivation;
2. to contain symptoms in soils already infected by *M. cannonballus* with sustainable control strategies.

Integrated prevention system of melon collapse

An integrated strategy for the disease management in new greenhouse cultivation need to built up in order to prevent the spread of *M. cannonballus.*

Fertility and soil health have to be maintained, thus favouring natural suppressiveness. Therefore farmers have to develop tools (organic amendments, rotation and tillage) enabling one to manage soil biotic and abiotic factors in order to increase soil suppressiveness to diseases (Janvier *et al*., 2007).

In melon greenhouse cultivation, a strategy based on inter-crops with legumes and grasses has to introduced, avoiding monoculture. This should favour the establishment of a complex of soil beneficial microorganisms, including endomycorrhize and *Trichoderma* species, similarly to uncultivated soils.

Furthermore, it is necessary to carry out a phytopathological analysis of plantlets from nursery in order to prevent the introduction of the pathogen in the greenhouses.

An effective implementation could be obtained by transplanting mycorrhized certified seedlings, which increase on one hand the plant nutrition, on the other the resistance level toward melon collapse. Tarbell and Koske (2007) suggested that commercial AMF inocula will vary greatly in their ability to roots colonization in different growing mixes and with different host plants. The results obtained in Chapter 6 provide a simple protocol, which can be easily introduced in the nursery production. It has to be underlined that the use of mycorrhized plants has to be associated with a change in the fertilisation protocols, such as the use of chemical fertilisers with a low content of phosphorus or a complete substitution of chemical fertilisers with organic matter.

The success of a biological control programme relies in the good adaptation of a given beneficial micro-organism to the local environmental conditions in which it is supposed to work (Cordier and Alabouvette, 2009). Thus, the selection of an endo-mycorrhizal species should take into account the efficacy toward the target pathogen along with the conditions where the mycorrhiza should develop. To be effective, the mycorrhizal species must to be established in the soil and in the rhizosphere of plants, therefore ecological fitness is an important trait. A method for obtaining effective mycorrhizal species is to select the candidate isolates from areas of the plant and soil where it is expected to function in disease control, and where it is growing under conditions of temperature, moisture, soil microflora composition and nutrient availability that approximate those found in nature.

Chemical soil treatments by fumigation have to be avoided because they are not selective, thus decreasing both pathogen and beneficial populations (Bendavid-Val *et al*., 1997). Moreover, fumigation, which is a high impact treatment will be probably banned in the next future within the “National Plans for the Integrated Control of pathogens”, that have to be introduced in the frame of the Directive **2009/128/CE.**

Solarization practice is a promising method to reduce populations of soilborne pests and weeds without the use of pesticides. However, the destruction of beneficial organisms such as arbuscular mycorrhizal fungi also may occur, thereby reducing positive effects of solarization (Schreiner *et al.*, 2001). Moreover, solarization has to be limited by the drawback regarding the disposal of the used plastic materials. Therefore, we recommend to integrated with the application of organic matter, such as compost or green manure, and the use of biodegradable plastic (Gamliel *et al*., 2000; Bonanomi *et al*., 2008).

To limit the phenomenon of the increase in salinity due to the fertirrigation, greenhouses have to be left uncovered at the time of replacement of the plastic film (every 2-3 years) to allow the leaching of salts favored by the autumn rains. Open field represent a soil environment suppressive to *M. cannonballus*. Therefore, it is convenient to mount moveable greenhouses or, if possible, move the greenhouses every 4-5 years, in order to cultivate melon in soils not previously cultivated with this crop.

Sustainable control strategies against M. cannonballus

Sustainable control strategies should be used on greenhouses where *M. cannonballus* has been previously monitored. The integrated approach described in the present work is designed to restore biological fertility and soil health. Taking together, research data indicate that it is possible to contain the disease in the absence of fumigation, which is completely incompatible in a sustainable farming system, as required by Directive **2009/128/CE** and that effectiveness of fumigation declines over time. In case, chemical fumigation can be substituted with bio-fumigation. However, incorporating *Brassica* spp. residue to reduce population of soil-borne pathogens should be assessed because contrasting results of effectiveness (Larkin and Griffin, 2005; Njoroge *et al*., 2008). Used integrated with others control strategies, biofumigation has potential for contributing to *M. cannonballus* management in melon greenhouse crops.

Solarization is a sustainable technique effective in soil-borne disease containment,

but it is ineffective toward melon collapse as reported in chapter 4. Therefore, to increase the effectiveness, this technique can be used only integrated with other sustainable treatments as biofumigation.

As indicated by data reported in Chapter 3, it is not suggested to transplant in greenhouse tolerant genotypes in order to avoid the increase of inoculum of *M. cannonballus* in soil.

Indeed, the root system of a tolerant plant is capable to supporting the production of approximately 400.000 ascospores (Waugh *et al*., 2003). Consequently, the fungus has great potential to increase in affected melon greenhouse. More late melon cultivars have to be chosen, alone or grafted on *Cucurbita* rootstocks. As reported in Chapter 4, *Cucurbita* rootstocks are fully resistant, however, resulting melons are characterised by lower quality compared to not-grafted ones. The sanitation practices based on the immediate removal of crop residues is an operation effective in decreasing inoculum in the soil (Radewald *et al*., 2004). As shown in this work, the use of compost is fully compatible with melon productivity by providing on one hand optimal nutrient supply for growth and production, on the other a significant reduction of melon collapse (Chapter 5).

It is more effective to supply compost at the end of the crop cycle to promote the suppressive action of the antagonists present in the amending. Thus, it is likely that the inoculum of the pathogen, which consists as ascospores, can be decreased over time by the action of the beneficial micro-organisms present in the amending. A future perspective is to use compost enriched with autochthonous *Trichoderma* species and other beneficial micro-organisms isolated from soil or rhizosphere from the same area of cultivation (Reda *et al*., 2008). The expectation is that native antagonistic species, alone or in combination, may have greater efficiency in terms of disease control, plant growth stimulation and adaptability compared to those obtained from areas with diverse environmental characteristics. All the described integrated control measures have to be implemented with a crop rotation plan, which ensure plant health and decrease of inoculum in the soil.

Conclusion

Collapse of melon occurs mainly in greenhouse cultivation, while it has been never reported in open field. Well-grown and productive crops are generally less susceptible to diseases. This also because rotation strongly soil sickness as well the establishment of inoculum of soil-borne pathogens. Protected cultivation is an extremely high-input procedure to obtain food and other agricultural products per unit of land. Moreover, crop protection activities contribute to the total input in a high proportion mainly through grafting and application of fumigants. Integrated sustainable practices can create a cultural environment similar to that of the open field. When sustainable measures are integrated with the use of resistant germplasm, and with biological control, a greatly reduced input and costs can be achieved. In these circumstances, growers are stimulated to make decisions based on economically founded criteria, moving toward an integrated view of plant health as a result of complex interactions between the plant, other organisms and the physical and chemical environment.

In order to achieve an integrated strategy for the sustainable control of melon collapse, the present research has generated the following results:

- melon genotypes susceptible and resistant to collapse have been identified;
- ineffective (solarization, grafting on melon hybrids), to be excluded (fumigation) or effective (grafting on *Cucurbita* species) disease control systems have been identified;
- a mycorrhization protocol useful in nursery to obtain mycorrhized plantlets to be used within a sustainable collapse management has been set up;
- a protocol for the greenhouse-use of compost characterized by suppressiveness ability in soil subjected to intensive agriculture has been set up;
- a system of integrated and sustainable disease management of collapse of melon immediately applicable in the territory, has been described.

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