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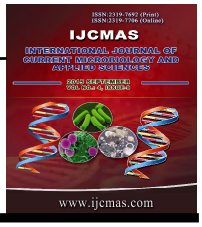

Original Research Article

Effects of Crop Sequences on Soil Population Dynamics of *Monosporascus cannonballus* Ascospores and *Monosporascus* Root Rot and Vine Decline Incidence

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Keywords

Crop sequence, Ascospores densities, Incidence of MRRVD, *M. cannonballus*

A B S T R A C T

Crop sequences effect on the soil population dynamics of *Monosporascus cannonballus* ascospores and the incidence of *Monosporascus* root rot and vine decline (MRRVD), was investigated in a field in which three different cucurbit crops: melon, watermelon, and watermelon grafted onto *Cucurbita* rootstock, and tomato, were grown during two consecutive growing seasons. Cultivation of melon or watermelon crops in the first growing season resulted in an increase of soil ascopore densities. But, on the contrary, the soil ascospore densities in the second growing season were lower when grafted watermelon or tomatos were cultivated in the first growing season. In the second growing season, MRRVD incidence for each cucurbit crop was significantly different depending on the previous crop, being in general higher when melon or watermelon were the previous crops, slightly lower when the previous crop was grafted watermelon and the lowest when the previous crop was tomato. Disease incidence corresponded with the percentage of isolation of *M. cannonballus* from the roots, being always significantly lower when the previous crop was tomato. These results demonstrate the potential of crop rotation as a management strategy to reduce infection and reproduction of *M. cannonballus*, ascospore densities in soil and disease incidence in cucurbits.

Introduction

Monosporascus root rot and vine decline (MRRVD) is one of the most destructive cucurbit diseases worldwide, causing significant yield losses on melon (*Cucumis melo* L.) and watermelon (*Citrulluslanatus* (Thunb.) Matsum and Nakai) crops (Cohen *et al.*, 2012; Martyn and Miller, 1996). The disease is caused by the soilborne ascomycetes *M. cannonballus* (Pollack and Uecker), currently reported in 20 countries (Cohen*et al.*, 2012; AlMawaali *et al*,. 2013), and *M. eutypoides* (Petrak) von

Arx, recently found as another cause of MRRVD in Tunisia (Ben Salem *et al.,* 2013). Aboveground symptoms of MRRVD are noticeable on the vines of melon and watermelon plants, which wilt and collapse rapidly at the end of the growing season, some weeks prior to harvest. They are the consequence of the gradual destruction of the root systems, which become necrotic with lack of the secondary and tertiary feeder roots, after the infection by *Monosporascus* spp. (Cohen *et al.,* 2012; Martyn and Miller, 1996). *Monosporascus cannonballus* and *M. eutypoides* are monocyclic pathogens. In affected cucurbit roots both pathogens produce fertile perithecia containing ascopores, which are the only known inoculum source (Cohen *et al.,* 2012; Martyn and Miller1996). Control of soil borne plant pathogens is problematic and the management of MRRVD is currently based on integrating differentstrategies, with varying degrees of success (Cohen *et al.,* 2012). Among them, chemical control combined with soil solarisation with fumigants at reduced dosage has proved its effectiveness to reduce the incidence of *M. cannonballus* on melon crops in Israel and United States (Cohen *et al.,* 2000; Stanghellini*et al.,* 2003).In Israel, post-planting application of fungicides was evaluated in field trials, which confirmedthe efficacy of fluazinam to control *M. cannonballus* (Cohen *et al.,* 1999). In subsequent research, azoxystrobin, fluazinam, prochloraz and pyraclostrobin+boscalid were also highly effective in the control of MRRVD (Pivonia *et al.,* 2010). However, these practices can be expensive and the range of authorized products is becoming limited. Grafting melon and watermelon onto *Cucurbita* hybrid rootstocksis a promising management strategy against MRRVD (Cohen *et al.,* 2007; Cohen *et al.,* 2000; Cohen *et al.,* 2012). Although *Cucurbita* hybrids are not completely resistant to *M. cannonballus*, they provide satisfactory control and reduce crop losses (Beltran *et al.,* 2008; Mertley *et al.,* 1993b). In general, grafted plants are more vigorous and productive, showing an improved agronomic performance due to a better tolerance to diverse environmental stresses, such as soil type, high soil salinity, low temperatures and drought (Louws *et al.,* 2010; Lee *et al.,* 2010). Grafting cucurbits onto *Cucurbita* hybrid rootstocks, alone or in combination with soil disinfectants, is used routinely by cucurbit growers (Beltran *et al.,* 2008; Cohen *et al.,* 2005; Edelstein *et al.,* 1999; Pivonia *et al.,* 1996). In Tunisia, watermelon grafting was first used against Fusarium wilt (Boughalleb *et al.,* 2008). Later, Jebari *et al.* (2009) studied the tolerance of some commercial cucurbit rootstocks against *M. cannonballus* in greenhouse conditions and, currently, watermelon plants grafted onto *Cucurbita* rootstocks are progressively replacing watermelon crops in the main Tunisian cucurbit production regions. In cucurbit crops, the increased incidence and severity of soilborne pathogens is the result of the sum of several cultural factors such as the use of hybrid cultivars, transplanted seedlings, plastic mulch, drip irrigation, increased plant density and excessive fruit load(Bruton1998;Cohen*et al.,* 2012).More specifically, Bruton (1998) pointed out that for MRRVD, inadequate crop rotation has likely contributed more than other factors to the severity of this disease by building-up the inoculum of *M. cannonballus* in soil. Beneficial effects of crop rotation compared to monoculture have been reported to include improvements in soil moisture, soil nutrients, soil structure and soil fungus populations (Griffith *et al.,* 1988; Liebman andDyck 1993; Peterson and Vargel 1989; Roder *et al.,* 1989; Williams and Schmitthenner, 1962). The abandonment of crop rotation could result in an increase of

M. cannonballus ascospore populations in soil (Beltran *et al.*, 2008), but the effects of crop sequences have never been investigated as a potential strategy for managing MRRVD. Thus, the main objective of this work was to determine the effects of different crop sequences on the soil population dynamics of *M. cannonballus* ascospores and the incidence of MRRVD, in a field in which three different cucurbit crops: melon, watermelon, and watermelon grafted onto *Cucurbita* rootstock, and tomato, were grown during two consecutive growing seasons.

Materials and Methods

Experimental plot

The study was performed in an experimental plot with a total area of 693m^2 located in a field belonging to the farm of the High Institute of Agronomy (ChottMariem, Sousse, Tunisia). This plot had been used for cucurbits cultivation in previous years and it had a history of MRRVD. Trials were conducted during two consecutive growing seasons in 2010 and 2011. In each season, three cucurbit crops (melon cv. Afamia, watermelon cv. Sentinel, and watermelon cv. Sentinel grafted on *Cucurbita maxima* × *C. moschata* rootstock cv. Strongtoza), and tomato cv. Giganti, were grown. In 2010, the experimental plot was subdivided into three blocks (repetitions). In each block, four crops each distributed in four different subplots were planted (Table 1). Each subplot had an area of 6.4 m^2, and included two rows of 5 plants each. The plants were transplanted on 01 June onto raised beds spaced 1.6 m from center to center, with an in-row spacing of 0.80 m. The experimental design consisted of randomized blocks with three replicates for each of the tested cucurbit crops. In 2011, the plants were transplanted also on 01 June but, in each block the four different subplots per crop were distributed taking into account the crop planted in the previous season, with the objective to have all possible combinations leading to crop sequence (Table 1). In both growing seasons, the experimental field was drip irrigated and standard cultural practices were employed. In the first growing season, roots of the crops were left in the field after crop termination.

Soil sampling and ascospore quantification

In both years, soil samples were taken at four different moments during the growing season from planting (01 June) to harvest time (August). In 2010 (first growing season), soil samples were taken at 17, 49, 74 and 85 days after transplanting (sampling moments 1, 2, 3 and 4, respectively). In 2011 (second growing season), soil samples were taken at 17, 47, 72 and 87 days after transplanting (sampling moments 1, 2, 3 and 4, respectively). In both seasons the last sampling moment coincided with the harvest. In each experimental subplot and sampling moment, three soil samples were taken adjacent to the first row of plants between beds. Samples of approximately 100 g were taken randomly with a soil probe at a depth of 10 to 20 cm (Mertley *et al.*, 1993a). Soil samples were air dried at room temperature and sifted through a 2-mm sieve to remove soil clods prior to processing. Ascospores of *M. cannonballus* were extracted by a method adapted from Stanghellini and Rasmussen (1992). Each sample was passed through a 250µm sieve. A 20g subsample was mixed with 200 ml of water, agitated on a magnetic stirrer for 5 min, and washed through nested 75 and 30µm sieves. The material retained on the 30µm sieve was centrifuged at 2.000×*g* for 4 min. The supernatant was discarded and the pellet was resuspended in 30 to 40 ml of

50% sucrose and centrifuged for 2min at 2.000×*g*. Ascospores and other materials floating or suspended in the sucrose were decanted onto the 30µm sieve and washed into a clean centrifuge tube. A second sucrose extraction was performed on the residual soil pellet to salvage spores not recovered during the first extraction. The resulting suspension from the second extraction was added to the first, and the combined suspension was stored in a small quantity of water at 4°C until analyzed. Ascospores were enumerated in the water under a stereomicroscope (Nikon SMZ1000) at a magnification of ×60.

Disease assessment and isolation of *M. cannonballus*

In both years, in each subplot, plants located in the first row were used for disease assessment. The number of symptomatic plants and the total number of plants evaluated in each plot were used to calculate the incidence of MRRVD (in percent). At the end of each growing season (sampling moment 4), plants were carefully removed, and the root system was gently washed in tap water. Roots were inspected visually for evidence of root necrosis, and the observation of roots bearing perithecia of *M. cannonballus* containing single-spored asci. In addition, for isolation, small root fragments were surface sterilized for 1 min in a sodium hypochlorite solution (1.5% active chlorine) and washed twice with sterile water. Root fragments from discoloured areas of tissue were transferred onto Potato Dextrose Agar (PDA) (Biokar-Diagnostics, Zac de Ther, France) containing streptomycin sulfate (Sigma-Aldrich, Madrid, Spain) (PDAS) at 0.5 mg $ml^{-1}$and incubated in darkness at 25°C. In all, 21 root fragments per plant (3 Petri dishes containing 7 root fragments each) were prepared. Plates were examined daily for fungal growth during 7 days, and hyphal tips from all colonies were transferred to PDA and V8 juice agar for subsequent growth and sporulation. ***M. cannonballus*** was identified morphologically by the formation of perithecia containing only one large (rarely two), spherical ascospore per ascus. For microscopic observations, single perithecia were mounted in 100% v/v lactic acid and observed using a Zeiss Axio Scope A.1 microscope. For molecular confirmation, fungal mycelium was obtained from pure cultures grown on Potato-Dextrose-Broth (PDB) (Sigma-Aldrich, Steinheim, Germany) for three weeks at 25°C in the dark. The mycelium was mechanically disrupted by grinding it to a fine powder under liquid nitrogen using a mortar and pestle. Total DNA was extracted using the EZNA Plant Miniprep Kit (Omega Bio-tek, Norcross, GA).The ITS nr DNA region of the isolates was amplified using the universal primers ITS1F and ITS4. The PCR reaction mix was adjusted to a final volume of 25 µl with water (Chromasolv Plus, Sigma-Aldrich). PCR products were purified and sequenced in both directions by the DNA Sequencing Service Macrogen Inc., Sequencing Center (Seoul, South Korea).Sequences were edited using the Sequencher software (Version 5.0, Gene CodesCorporation, Ann Arbor, MI) and subjected to a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/).

Data analyses

The counts of symptomatic plants and data on *M. cannonballus* isolation at the end of the second growing season were used to compare MRRVD incidence (%) and the percentage of isolation of *M. cannonballus* among the three cucurbit crops evaluated, depending on the crop planted in the previous year of the sequence, using one-way analysis of variance (ANOVA). Means were compared by the least significant difference test (p<0.05). Analyses were

performed using the software Statgraphics Plus 5.1 (Manugistics Inc., Rockville, MD, USA), the significant interaction between crops and treatments was performed using STATIX 9.0 for each parameter.

Results and Discussion

Effects of crop sequence on the soil population dynamics of *M. cannonballus* ascospores and the incidence of MRRVD

In both growing seasons (2010 and 2011), the only *Monosporascus* species isolated from the roots of plants showing symptoms of MRRVD was *M. cannonballus* and confirmed by molecular tools.The four crops behave differently according to the parameters ($p < 0.05$). Analysis of variance showed a significant difference in disease incidence between the four sampling moments ($p < 0.05$) and the four crop sequences ($p < 0.05$). This effect was revealed on the expression of the disease mainly in the roots and leaves throughout the life cycle of the plant unlike the ascospores densities; this is due to the soil complexity and the initial source of inoculum of *M. cannonballus* was unknown (Table 2). According data analysis, the different interactions like sampling moments * treatments (Figure 2A), sampling moments* crops (Figures 1B, 2D) and experiments * crops (Figures 1C, 2E) exhibit a significant effect on ascopores densities and Disease incidence ($p<0.05$).

Sampling moments were representative, this trial was conducted in two years (2010 and 2011) noted an interaction with the other factors confirming that the different crop sequence may give a clear view on the impact of rotation(Figure 1). The initial (16DAP) and final ascospores population (harvest moment) on soil is higher at 2010 than 2011 (Figure 2).

Melon as previous crop

In the subplots in which the first crop was melon (Figure 3), MRRVD incidence in 2010 increased progressively during the growing season reaching a level that ranged from 63.31 to 65.28% at the harvest moment. Ascospore densities in these subplots in the first sampling moment ranged between 4.12 and 5.57 asc/g of soil and, in general, these levels decreased, ranging from 2.13 to 2.52 asc/g of soil at the harvest moment. At the end of the growing season, the isolation of *M. cannonballus* from melon roots ranged from 31.54 to 36.11% of the root segments, and perithecia were observed in most of the subplots (Table 3). In 2011, when the crop sequence was established, the incidence of MRRVD was also very high for all cucurbit crops reaching values of 64.87% for melon, 66.00% for watermelon and 59.67% for grafted watermelon, but MRRVD was not observed on tomato plants (Figure 3). In general, during this second growing season, an increase of ascospore numbers was noticed for all crops in comparison with those recorded in 2010. The maximum values of ascospore densities were 6.60 and 7.20 asc/g of soil at sampling moment 3 for melon and watermelon, respectively, and 6.77 and 5.60 asc/g of soil at sampling moment 2 for grafted watermelon and tomato, respectively. At the end of the growing season, *M. cannonballus* was isolated from the roots of cucurbit crops with values of 34.52% (melon), 43.45% (watermelon) and 26.44% (grafted watermelon), but it was not isolated from tomato roots. Moreover, perithecia of the pathogen were observed only in cucurbits (Table 3).

Watermelon as previous crop

In the subplots in which watermelon was planted as the first crop (Figure 4), in 2010

MRRVD incidence at harvest ranged from 57.32 to 69.86%. In general, ascospore densities in these subplots showed an increase from the first and second samplings to harvest time, ranging at this moment from 2.27 to 6.03 asc/g of soil. At the end of the growing season, the isolation of *M. cannonballus* from watermelon roots ranged from 43.84 to 65.08% of the root segments, and perithecia were observed in diseased roots in all subplots (Table 3). In 2011, when the crop sequence was established, the incidence of MRRVD at harvest moment was also very high for all cucurbit crops, being of 73.98% for watermelon, 73.83% for melon and 57.86% for grafted watermelon (Figure 4), but MRRVD was not observed on tomato plants. During this growing season, ascospore numbers were very variable for all crops. The values of ascospore densities ranged from 0.53 to 3.30 asc/g of soil in watermelon, from 3.62 to 5.68 asc/g of soil in melon, and from 2.80 to 5.90 asc/g of soil in grafted watermelon. In tomato, the values ranged from 3.72 to 6.03 asc/g of soil. At the end of the growing season *M. cannonballus* was isolated from the roots of all cucurbit crops with values of 30.75 % (melon), 66.92 % (watermelon) and 13.69 % (grafted watermelon), but not from tomato roots. Perithecia of the pathogen were observed only in watermelon and melon roots (Table 3).

Grafted watermelon as previous crop

In the subplots in which the first crop was grafted watermelon (Figure 5), in 2010 MRRVD incidence ranged from 44.67 to 49.72% at the harvest moment. The ascospore densities in these subplots ranged from 1.88 to 3.88 asc/g of soil, and the isolation of *M. cannonballus* from the roots of the *Cucurbita* rootstock at the end of the growing season showed values ranging from 14.88 % to 22.81% of the root segments. Perithecia were observed in most of the subplots (Table 3). In 2011, when the crop sequence was established, MRRVD incidence reached a level of 55.17% for melon, 56.37% for watermelon and 56% for grafted watermelon (Figure 5), but MRRVD was not observed on tomato plants. During this second growing season, a decrease of ascospore numbers was noticed for all crops in comparison with those recorded in 2010. The maximum values of ascospore densities were 1.72 asc/g of soil for grafted watermelon, 2.25 asc/g of soil for watermelon, 2.47 asc/g of soil for melon, and 2.03 asc/g of soil for tomato. At the end of the growing season, the pathogen was isolated from the roots of cucurbit crops with values of 24.55% (melon), 24.33% (watermelon) and 24.8% (grafted watermelon), but not from tomato roots. Perithecia of *M. cannonballus* were observed only in watermelon and melon roots (Table 3).

Tomato as previous crop

In the subplots in which tomato was planted as the first crop (Figure 6), MRRVD was not observed in 2010. Ascospore densities in these subplots increased progressively during the growing season, reaching a maximum at the harvest moment. At the end of the growing season, *M. cannonballus* was not isolated from tomato roots and perithecia were not observed (Table 3). In 2011, when the crop sequence was established, MRRVD incidence for all cucurbit crops reached a level of 44.73% for melon, 46.35% for watermelon, and 39.18% for grafted watermelon (Figure 6). The values of ascospore densities ranged from 1.10 to 1.90 asc/g of soil for melon; 0.90 to 3.38 asc/g of soil for watermelon; 1.25 to 1.78 asc/g of soil for grafted watermelon; and from 0.88 to 6.65 asc/g of soil for tomato. At the end of the growing season, *M. cannonballus* was isolated only from cucurbit crops with a level of 11.82% for melon, 12.6% for

watermelon and 10.93% for grafted watermelon, but it was not isolated from tomato roots, and perithecia were not found in any crop (Table 3).

Table 4 shows the statistical comparison of MRRVD incidence, and the percentage of isolation of *M. cannonballus* at the end of the second growing season among the three cucurbit crops evaluated, depending on the crop planted in the previous season of the crop sequence. In melon there were significant differences in MRRVD incidence and the percentage of isolation depending on the previous crop (p=0.0011 and p=0.002, respectively), being the values of both variables the highest when melon and watermelon were the crops planted in the previous growing season, intermediate if the previous crop was grafted watermelon and the lowest when the previous crop was tomato.

Identical effect was noticed on watermelon, with significant differences in MRRVD incidence and the percentage of isolation of *M. cannonballus* depending on the previous crop (p=0.0027 and p=0.0036, respectively), being always tomato the previous crop that resulted in the lowest values for both disease incidence and percentage of isolation. In grafted watermelon there were also significant differences in MRRVD incidence and the percentage of isolation depending on the previous crop (p=0.0054 and p=0.0018, respectively), but in this case MRRVD incidence was not significantly different among melon, watermelon and grafted watermelon as the previous crops, being tomato the crop that resulted in the lowest value, being significantly different from the cucurbits.

In this study, we found evidences of the positive and negative effects of different crop sequences, established with three cucurbit crops and tomato, on the soil population dynamics of *M. cannonballus* ascospores and the incidence of MRRVD during two consecutive growing seasons. Although ascospore quantification showed a high degree of variability, in general, when melon or watermelon crops were cultivated in the first growing season, this resulted in an increase of soil ascopore densities in the second growing season.

Melon and watermelon have been reported as highly susceptible hosts for *M. cannonballus*, which is able to colonize extensively their roots systems (Mertely*et al.*, 1993b; Martyn and Miller 1996), favouring the production of a high number of perithecia and ascospores, which occurs primarily at the end of the growing season, especially after crop termination (Stanghellini *et al.*, 2004; Waugh *et al.*, 2003). Beltrán *et al.* (2008) reported a remarkable increase of ascospore counts in melon and watermelon crops at the end of the growing season in a parallel way to the quick development of MRRVD symptoms, leading to plant death. On the contrary, in general, the soil ascospore densities in the second growing season decreased when grafted watermelon or tomato were cultivated in the first growing season. Both crops were evaluated in the host range study of *M. cannonballus* conducted by Mertely *et al.* (1993b), where *Cucurbita* spp. [*C. maxima* Duchesne, *C. moschata* (Duchesne) Duchesne ex Poir., and *C. texana*A. Gray] were rated as less susceptible to the pathogen than melon or watermelon, although the pathogen was capable of penetrating the roots and reproducing. In the same study, tomato was considered resistant because the fungus was not isolated from inoculated plants, and failed to produce perithecia.

Table.1 Distribution of the experimental subplots corresponding to the four crops evaluated (melon, watermelon, grafted watermelon and tomato) in the experimental plot during two consecutive growing seasons (2010 and 2011)

BLOCK	EXPERIMENTAL PLOT 2010							
	SUBPLOTS[a]							
1	WA	ME	GRWA	WA	TO	ME	TO	WA
	GRWA	TO	GRWA	WA	ME	ME	GRWA	TO
2	TO	GRWA	ME	WA	WA	ME	TO	GRWA
	WA	WA	GRWA	TO	ME	GRWA	TO	ME
3	ME	WA	TO	ME	ME	WA	GRWA	TO
	WA	GRWA	ME	WA	GRWA	TO	TO	GRWA
BLOCK	EXPERIMENTAL PLOT 2011							
	SUBPLOTS							
1	WA	ME	WA	TO	WA	TO	GRWA	GRWA
	GRWA	TO	TO	ME	WA	GRWA	ME	ME
2	GRWA	TO	WA	ME	TO	TO	ME	WA
	GRWA	WA	GRWA	TO	ME	ME	WA	GRWA
3	TO	ME	WA	WA	ME	GRWA	GRWA	TO
	WA	TO	GRWA	TO	WA	ME	GRWA	ME

[a]WA= Watermelon, ME= Melon, GRWA= Grafted Watermelon, TO= Tomato

Table.2 Analysis of variance for the effects of crop, experiment, sampling moments and treatments on ascospore densities (asc/g of soil) and disease incidence (%)

	df[a]	Ascospore densities (asc/g of soil)		Disease incidence (%)	
		MS[b]	P <F[c]	MS[b]	P <F[c]
Crop	3	0,2104	<0,0001	1,2624	<0,0001
Experiment	1	0,0399	0,0029	0,0890	<0,0001
Sampling moments	3	0,0026	0,5515	3,5274	<0,0001
Treatments	3	0,0237	0,0004	0,5605	<0,0001
Crop*experiement	3	0,0780	<0,0001	0,4333	<0,0001
Sampling moments*crop	9	0,0365	<0,0001	0,1536	<0,0001
Treatments*crop	9	0,0148	<0,0001	0,0052	0,4360
Experiement*treatments	3	0,0040	0,3602	0,5900	<0,0001
Sampling moments * treatments	9	0,0055	0,1563	0,1026	<0,0001
Experiment * sampling moments	3	0,0045	0,3165	0,0299	0,0008

[a] Degrees of freedom, [b] Mean square, [c] Probabilities associated with individual F tests.

Table.3 Mean percentage of isolation of *Monosporascus cannonballus* from the roots of the different crops evaluated at the end of each growing season (2010 and 2011) and presence or absence of perithecia

2010			2011		
Crops[a]	**Isolation %**	**Perithecia**	**Crops**	**Isolation %**	**Perithecia**
Melon	31.54[b]	-[c]	Melon	34.52	+
Melon	36.11	+	Watermelon	43.45	+
Melon	35.11	+	Grafted watermelon	26.44	+
Melon	34.72	+	Tomato	0.0	-
Watermelon	48.02	+	Melon	30.75	+
Watermelon	49.00	+	Watermelon	66.92	+
Watermelon	43.84	+	Grafted watermelon	13.69	-
Watermelon	65.08	+	Tomato	0.0	-
Grafted watermelon	16.27	-	Melon	24.55	+
Grafted watermelon	22.81	+	Watermelon	24.33	+
Grafted watermelon	21.82	+	Grafted watermelon	24.80	-
Grafted watermelon	14.88	+	Tomato	0.0	-
Tomato	0.0	-	Melon	11.82	-
Tomato	0.0	-	Watermelon	12.60	-
Tomato	0.0	-	Grafted watermelon	10.93	-
Tomato	0.0	-	Tomato	0.0	-

[a]Crops are shown according to the different crop sequences established in the subplots.
[b]Percentage of isolation of M. cannonballus from root segments incubated on PDAS at 25°C. Mean of three repetitions of 21 infected root segments. [c]Presence or absence of perithecia in the root systems (roots of three plants per crop were observed)

Table.4 Comparison of *Monosporascus* root rot and vine decline incidence, and the percentage of isolation of *Monosporascus cannonballus* at the end of the second growing season among the three cucurbit crops evaluated (muskmelon, watermelon and grafted watermelon), depending on the crop planted in the previous season of the crop sequence

		Second growing season	
Crop	**Previous crop**	**Disease incidence%**[a]	**Isolation%**[b]
Muskmelon	Muskmelon	64.87 b[c]	34.52 a
	Watermelon	73.83 a	30.75 b
	Grafted watermelon	55.17 c	24.55 c
	Tomato	44.73 d	11.82 d
Watermelon	Muskmelon	66.00 b	66.92 a
	Watermelon	73.98 a	43.45 b
	Grafted watermelon	58.37 c	24.33 c
	Tomato	46.35 d	12.60 d
Grafted watermelon	Muskmelon	59.67 a	26.44 a
	Watermelon	57.86 a	13.69 b
	Grafted watermelon	56.00 a	24.80 a
	Tomato	39.18 b	10.93 b

[a] Percentage of plants showing symptoms of *Monosporascus* root rot and vine decline. Mean of three repetitions of 5 plants each.
[b] Percentage of isolation of *M. cannonballus* from root segments incubated on PDAS at 25°C. Mean of three repetitions of 21 root segments.
[c] ANOVA. Means in a column followed by the same letter are not significantly different according to Students least significant difference test at $p<0.05$.

Figure.1 Significant interactions carried out by STATIX 9.0 for Ascospores densities (asc/g of soil); A: crops*treatments; B: sampling moments*crops; C: experiments*crops

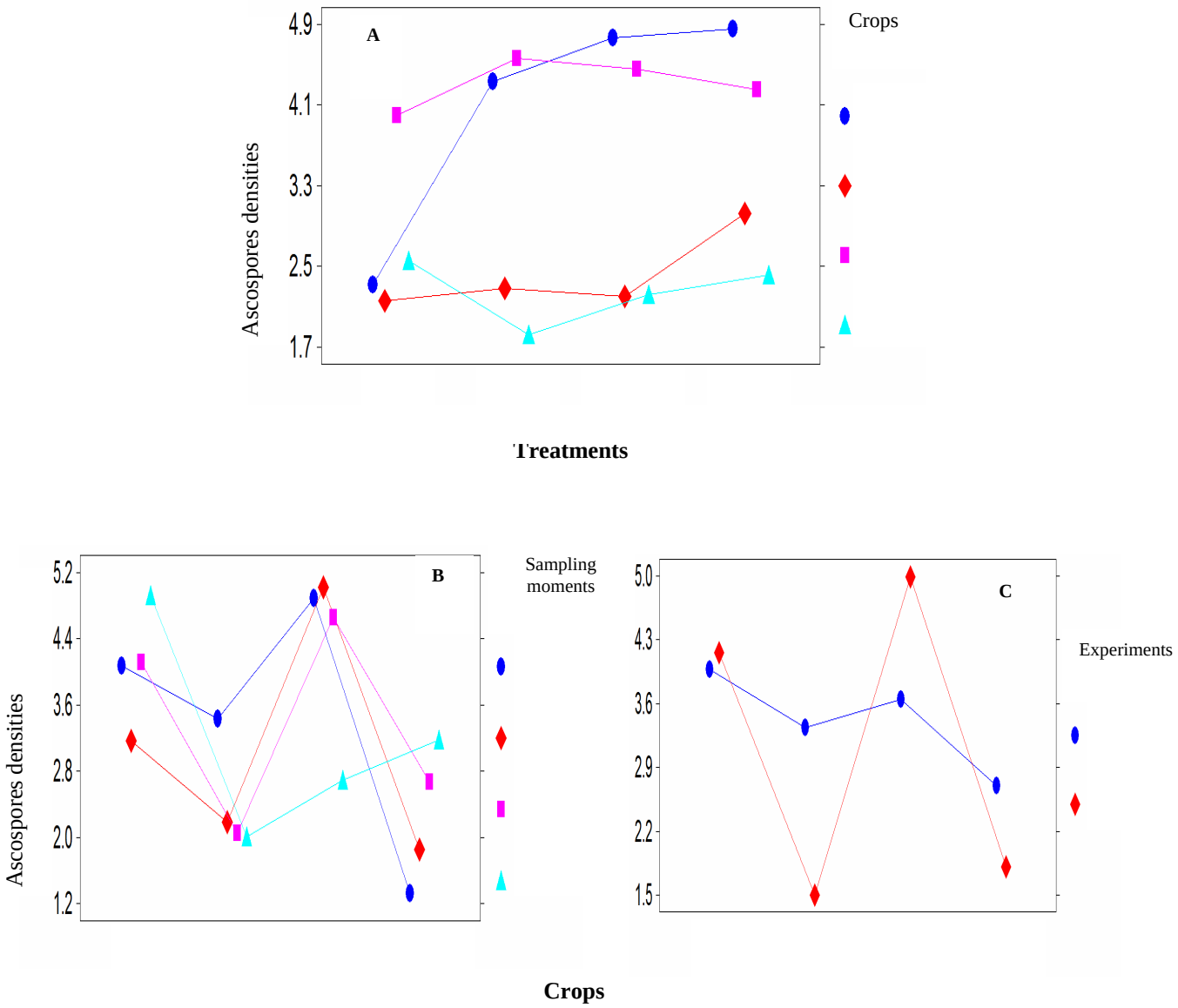


Figure.2 Significant interactions carried out by STATIX 9.1 for Disease incidence (%);A: sapmling moments*treatments, B: experiments*treatments; C: experiements*sampling moments; D: sampling moments*crops; E: experiements*crops

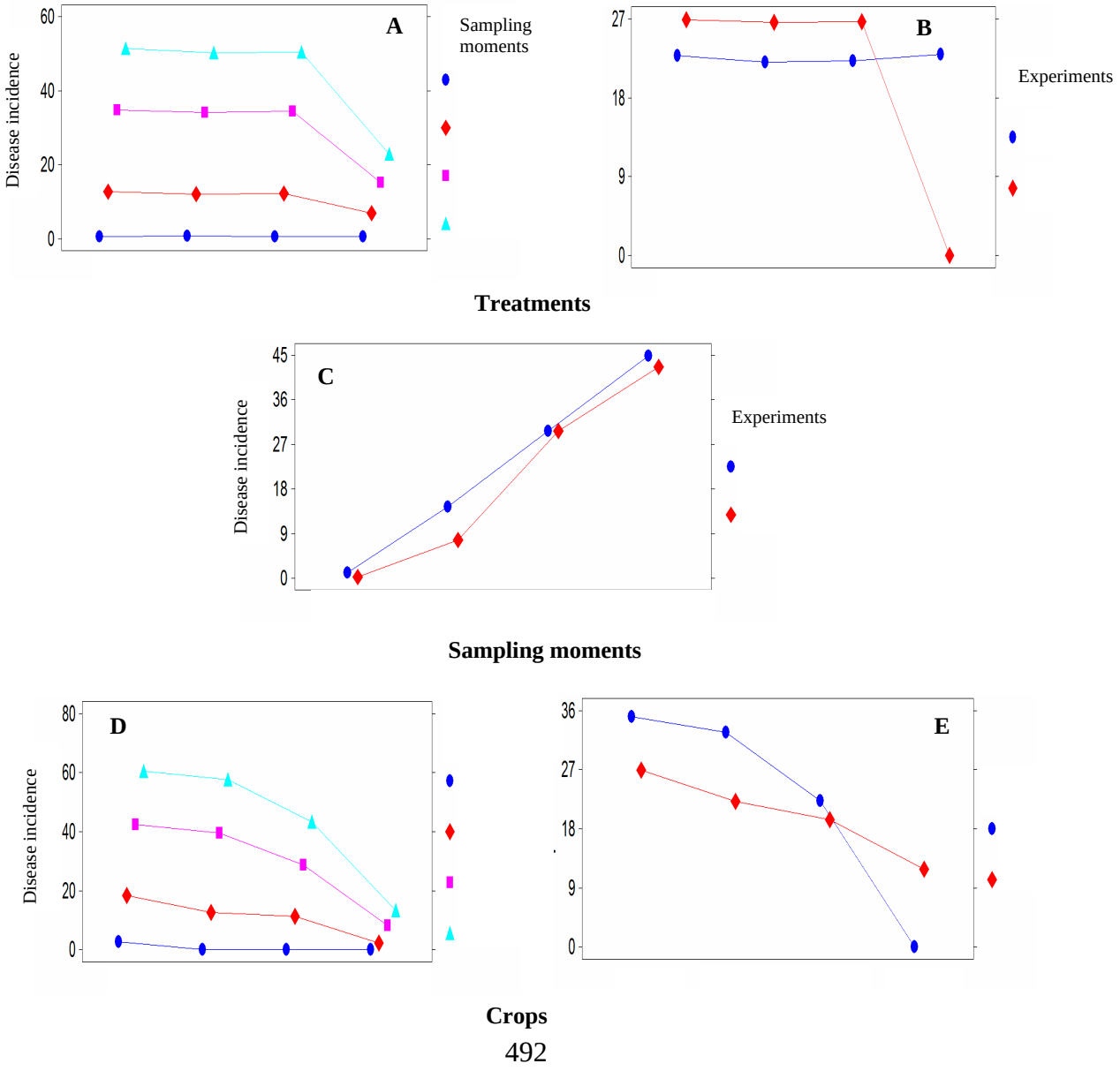


Figure.3 Population dynamics of *M. cannonballus* ascospores in soil (ascospores / g of soil) during two consecutive growing seasons (2010 and 2011), and *Monosporascus* root rot and vine decline incidence (% of symptomatic plants) for the different crop sequences in the subplots in which the crop planted in 2010 was melon. In each growing season ascospore density (mean ± standard error of three repetitions of three soil samples each) and symptomatic plants (mean ± standard error of three repetitions of five plants each) were evaluated at four different moments: in 2010, 17, 49, 74 and 85 days after transplanting; in 2011, 17, 47, 72, and 87 days after transplanting

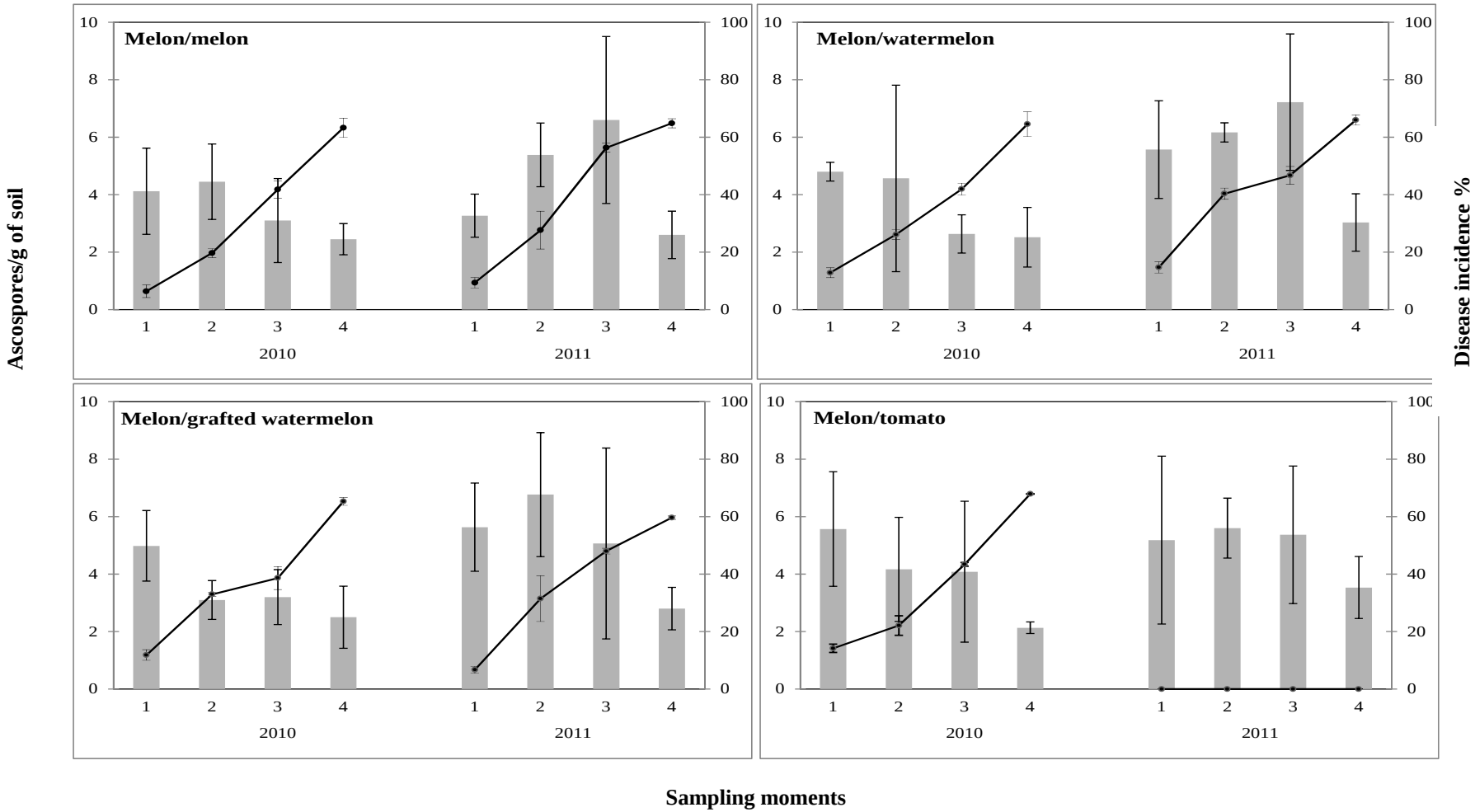


Figure.4 Population dynamics of *M. cannonballus* ascospores in soil (ascospores / g of soil) during two consecutive growing seasons (2010 and 2011), and *Monosporascus* root rot and vine decline incidence (% of symptomatic plants) for the different crop sequences in the subplots in which the crop planted in 2010 was watermelon. In each growing season ascospore density (mean ± standard error of three repetitions of three soil samples each) and symptomatic plants (mean ± standard error of three repetitions of five plants each) were evaluated at four different moments: in 2010, 17, 49, 74 and 85 days after transplanting; in 2011, 17, 47, 72, and 87 days after transplanting

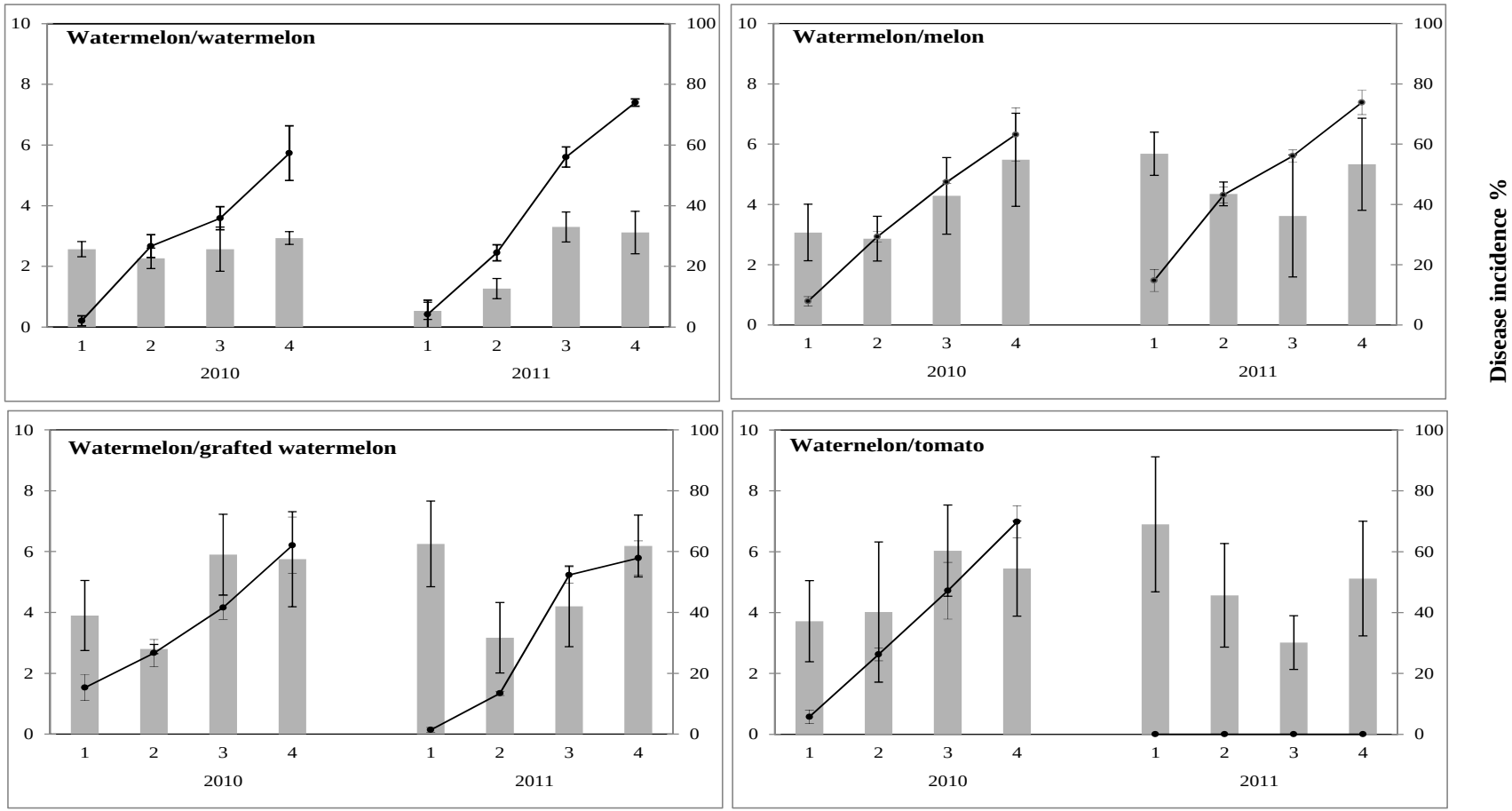


Figure.5 Population dynamics of *M. cannonballus* ascospores in soil (ascospores / g of soil) during two consecutive growing seasons (2010 and 2011), and *Monosporascus* root rot and vine decline incidence (% of symptomatic plants) for the different crop sequences in the subplots in which the crop planted in 2010 was grafted watermelon. In each growing season ascospore density (mean ± standard error of three repetitions of three soil samples each) and symptomatic plants (mean ± standard error of three repetitions of five plants each) were evaluated at four different moments: in 2010, 17, 49, 74 and 85 days after transplanting; in 2011, 17, 47, 72, and 87 days after transplanting

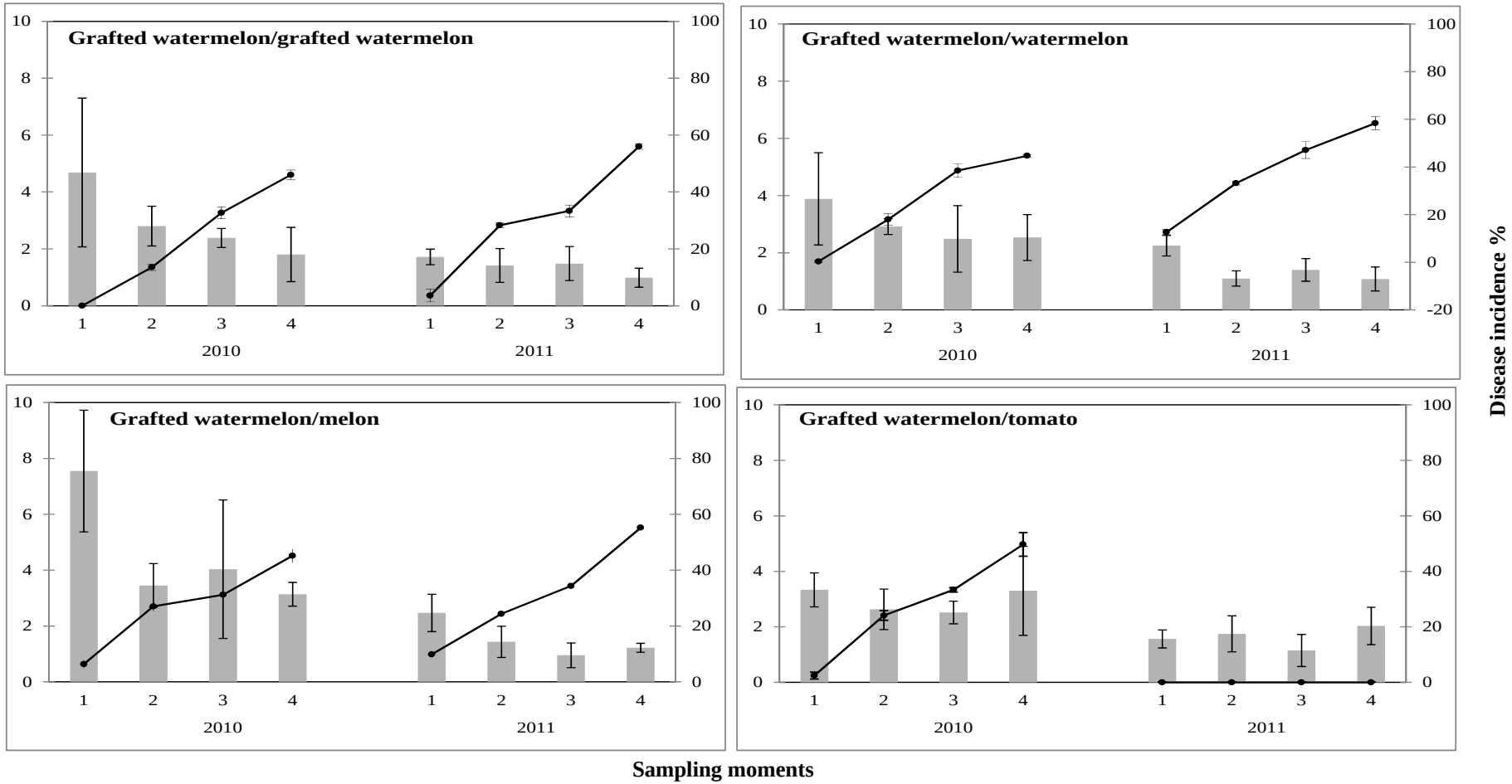
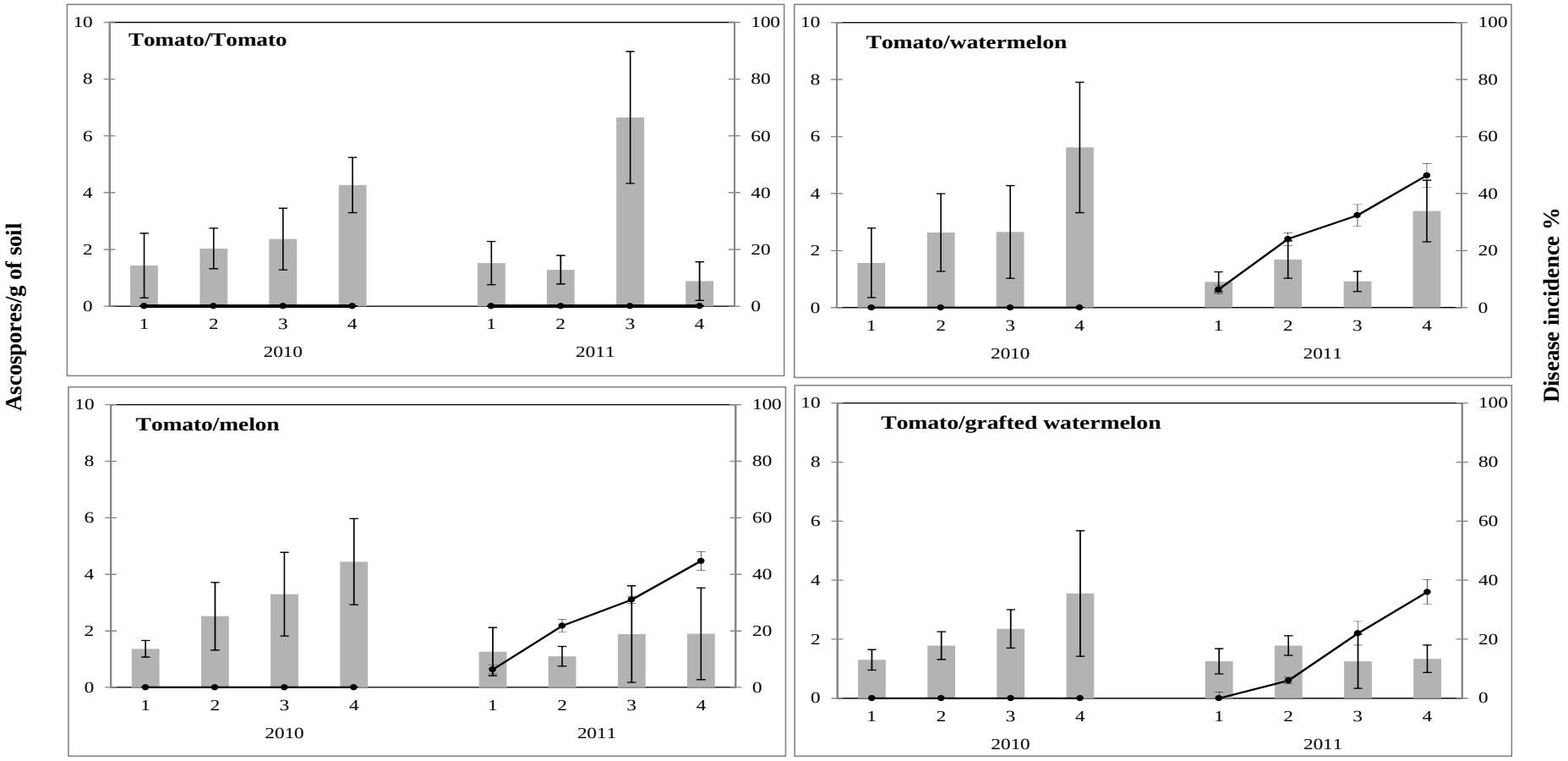


Figure.6 Population dynamics of *M. cannonballus* ascospores in soil (ascospores / g of soil) during two consecutive growing seasons (2010 and 2011), and *Monosporascus* root rot and vine decline incidence (% of symptomatic plants) for the different crop sequences in the subplots in which the crop planted in 2010 was tomato. In each growing season ascospore density (mean ± standard error of three repetitions of three soil samples each) and symptomatic plants (mean ± standard error of three repetitions of five plants each) were evaluated at four different moments: in 2010, 17, 49, 74 and 85 days after transplanting; in 2011, 17, 47, 72, and 87 days after transplanting



Moreover, Beltrán *et al.* (2008) in a study about the epidemiology of *M. cannonballus* in cucurbit fields in Spain, indicated that peritheciaof the fungus were not observed on roots of grafted watermelon crops, and ascospore soil populations remainedstable. An increase of ascospore counts was noticed in all tomato subplots, during the first growing season. *Monosporascus cannonballus* is considered a monocyclic pathogen and ascospores are considered the primary inoculum for initial infection (Cohen*et al.,* 2012; Martyn and Miller 1996). Nevertheless, inoculum of *M. cannonballus* (ascospores and mycelia) can have a saprophytic growth, being produced belowground in infected roots left in the field after crop termination (Cohen*et al.,* 2012; Stanghellini *et al.,* 2004). Thus, although the experimental plot had been in fallow during one year before the first growing season (2010), and no perithecia were observed on tomato roots, crop residues from previous cucurbits cultivation could have supported inoculum build-up in soil.

As expected, MRRVD incidence in the first growing season was higher on melon and watermelon crops than in grafted watermelon, and the disease was not observed in tomato. Nevertheless, the *Cucurbita* rootstock was not completely resistant to the disease and the infection of the roots by *M. cannonballus* was confirmed by isolation and the observation of perithecia in most of the subplots, also in the second growing season. Knowledge of the response of Cucurbita accessions to *M. cannonballus* is limited (Cohen *et al.,* 2012), and previous evaluations of *Cucurbita* rootstocks used for grafting melon and watermelon plants indicated considerable variation in the response to *M. cannonballus* (Mertely *et al.,* 1993b; Cohen *et al.,* 2005; Cohen *et al.,* 2007; Beltrán *et al.,* 2008). Interestingly, in the second growing season, MRRVD incidence for each cucurbit crop evaluated was significantly different depending on the previous crop, being in general higher when melon or watermelon were the previous crops, slightly lower when the previous crop was grafted watermelon and the lowest when the previous crop was tomato. Disease incidence corresponded with the percentage of isolation of *M. cannonballus* from the roots, being always significantly lower when the previous crop was tomato. This result could be explained by the increase in the soil populations of *M. cannonballus* in the first growing season due to the cultivation of very susceptible crops (melon and watermelon), that, as indicated by Waugh *et al.* (2003), results in a concomitant increase in disease incidence or severity in the next cucurbit growing season. These authors conducted a study of the reproductive potential of *M. cannonballus*, concluding that the reproductive capability of *M. cannonballus* provides an explanation for the increased prevalence/severity of the disease in commercial cucurbit fields, particularly those that have been sequentially cropped to melon. Most authors has reported the ascospores densities in melon (Stanghellini *et al.,* 1996; Merteley *et al.,* 1993b; Radewald *et al.,* 2004; Medeiros *et al.,* 2006b). Obtained results have reported that ascospores densities in watermelon is higher than in melon unlike the results found by Heo *et al.* (2001a and b) and Medeiros *et al.* (2008). Our results demonstrate the potential of crop rotation as a management strategy to reduce infection and reproduction of *M. cannonballus* in cucurbit roots, ascospore densities in soil and the incidence of MRRVD; specially after cropping non-host crops for *M. cannonballus*, such as tomato. The duration and the crops recommended for these rotations require further research. In addition, although cultivation of grafted

watermelon was beneficial in terms of the reduction of disease incidence and percentage of isolation of the pathogen in subsequent cucurbit crops, this study confirms that grafted watermelon alone is not sufficient to control MRRVD. In fact the *Cucurbita* rootstock used was susceptible to *M. cannonballus* and the damage caused by the pathogen was probably reduced due to the extensive root system produced by plants in this genus that supports vines and fruits of watermelon in spite of some infection (Cohen *et al.,* 2007; Beltran *et al.,* 2008; Cohen *et al.,* 2012). Consequently, as recommended by Cohen *et al.* (2012), an integrated approach using multiple techniques should be the most appropriate strategy to manage MRRVD.

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