Journal of Plant Pathology (2006), **88** (3), 253-261 *Edizioni ETS Pisa, 2006*

STRATEGIES FOR MANAGEMENT OF *SCLEROTIUM CEPIVORUM* BERK. IN GARLIC

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SUMMARY

The impact of incorporation of onion, broccoli or carrot crop residues, followed by soil solarization and by incorporation of chicken manure-*Trichoderma harzianum* (Ch-T) at garlic planting, on sclerotium density and viability of *Sclerotium cepivorum*, white rot incidence and garlic yield was evaluated in the field in a 3 x 3 x 2 factorial arrangement. Planting and incorporation of broccoli significantly reduced sclerotium density and viability (53 and 14%, respectively) and increased the yield of garlic (18%) compared with incorporation of onion (control). Soil solarization significantly reduced inoculum density (75%), viability (84%) and disease incidence (88%), and increased garlic yield by up to 152%, compared with non-solarized treatments. Incorporation of broccoli in combination with soil solarization was the strategy, which gave the greatest reduction in sclerotium density and viability, the lowest white rot incidence, and the highest yield of garlic.

Key words: crop residues, garlic, integrated control, *Sclerotium cepivorum*, solarization.

INTRODUCTION

Sclerotium cepivorum Berk., the causal agent of white rot, exclusively affects species of *Allium,* and is found in practically all regions where species of *Allium* are grown (Entwistle, 1990; Crowe *et al.*, 1993; Schwartz and Mohan, 1995). It can cause losses from 1 to 100% (Perez-Moreno *et al.*, 1998) and has caused great damage in diverse regions of Europe, Asia, Africa, North, Central, and South America, Australia and New Zealand (Schwartz and Mohan, 1995).

In Mexico the disease is reported in the states of Aguascalientes, Chihuahua, Guanajuato, Puebla, Jalisco, Morelos, Michoacan, Queretaro, Tlaxcala, and Zacatecas (Perez-Moreno *et al.*, 1998). In Guanajuato, the main producer of garlic and onion, inoculum densities reach up to 700 sclerotia kg^{-1} of soil, causing losses from 1 to 100% (Perez-Moreno *et al.*, 1998).

The control of *S. cepivorum* is difficult because it forms abundant sclerotia that can remain dormant for more than 15 years (Crowe *et al.*, 1993). Germination of the sclerotia is stimulated by root exudates of *Allium* species (King and Coley-Smith, 1968; Coley-Smith, 1990). Reduction of *Allium* white rot severity has been achieved by: use of fungicides (Delgadillo *et al.*, 2002), application of synthetic *Allium* oil (Coley-Smith, 1990; Delgadillo *et al.*, 2004), crop rotation, soil flooding (Banks and Edgington, 1989), addition of composts (Entwistle, 1990), solarization and mulching (Zavaleta-Mejía *et al.*, 1994; Delgadillo *et al.*, 2004), incorporation of cruciferous residues (Zavaleta-Mejía *et al.*, 1992b), and application of antagonists (Chet and Henis, 1985; Crowe *et al.,* 1993; Ulacio-Osorio *et al.*, 2004).

It has been suggested that remediation of highly infested soils and sustainable management of *Allium* white rot will only be achieved through a combination of strategies, which must begin several years (1 to 3, or more depending on the degree of soil infestation) before planting garlic or onion (personal communication, Zavaleta-Mejía, 2002). Therefore, the objective of this study was to evaluate the impact of combination of growing onion, broccoli or carrot crops followed by their incorporation, solarization, and application (at the time of planting garlic) of chicken manure-*Trichoderma harzianum* on the density and viability of sclerotia of *S. cepivorum*, the incidence of the white rot and the garlic yield.

MATERIALS AND METHODS

Location of the experiment. The experiment was carried out at El Colorado farm in Cortazar, Guanajuato, Mexico, in a field with a fine texture soil heavily infested with *S. cepivorum.* The field was not cropped with

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garlic or onion for 2 years; during this period, two applications of allyl disulphide were made by the grower (between the summer and the autumn of 2001). Our experiment was done in the period from December 2001 to March 2003 and involved three phases, during which a 3 × 3 × 2 factorial experiment (planting and incorporation of three crops × three periods of solarization x incorporation or not of chicken manure-*Trichoderma*) with three repetitions was established.

First phase: December 1, 2001 - April 2, 2002, experimental site (1080 m^2), with inoculum density averaging 0.43 sclerotia g^{-1} of soil. The site was divided into three plots of 360 m^2 each. In each, onion, broccoli or carrot crops were planted. Once these were established, each parcel was sub-divided into three sub-parcels of 120 m^2 and in each sub-parcel, three plots of 40 m^2 were marked out. Four months later, the crops were rotavated into the soil.

To evaluate the effect of cropping and crop incorporation on sclerotium density and viability, soil samples were taken at planting time and 1 week after rotavating. Samples were collected by taking 24 soil cores of approximately 70 g from 0 to 15 cm depth in a zigzag pattern over each experimental unit. The cores were combined to provide a single composite sample.

The soil was dried and, from each sample, three aliquots of 30 g were taken for extraction of sclerotia (Adams, 1979). The number of sclerotia in each aliquot was counted and the inoculum density for each experimental unit was recorded. The viability of sclerotia was determined by taking 30 sclerotia from each experimental unit from each treatment; the sclerotia were disinfested with 1% sodium hypochlorite for 1 min, rinsed with sterile distilled water and, after drying, were cracked and placed on cylinders of water agar (of approximately 1 cm diameter) contained in Petri dishes (one sclerotium per cylinder). The sclerotia were then incubated for 10 days at 15-18°C (Crowe *et al.*, 1980).

Second phase. One week after rotavation, the site was ploughed and irrigated to field capacity, taking care to maintain the integrity of the areas previously marked out and on which solarization treatments for 0, 40 and 90 days were established (May 02 - August 02, 2002). This established a 3 × 3 factorial experiment (the three previous cultures × three levels of solarization) in a completely randomized design, with threefold replication from establishment of the three main blocks in the first phase. Each solarized/unsolarized plot consisted of three rows, 12 m long, and those due to receive solarization treatment were covered with clear polyethylene of 20μm thickness. The soil temperature was recorded hourly with electronic sensors (data loggers, Tinytalk II®) buried at a depth of 15 cm in two solarized and two non-solarized plots. During the solarization period there was some rain. At the end of the 90-day solarization period, soil samples were taken from the central row of each plot, following the procedure described for the first phase. For the viability test, all of the sclerotia recovered from each plot were used.

Third phase. The garlic bulb-seed of cultivar HK3 was sown on September 4, 2002. At planting time, 10 kg of chicken manure (obtained from Bachoco Company, Celaya, Guanajuato) and 1000 g of ground corn-cob containing 31 × 10^6 conidia of *Trichoderma harzianum* g^{-1} of corn-cob (Ulacio-Osorio *et al.*, 2004) were mixed into the top 10 cm of soil over 6 linear meters of each row. The *T. harzianum* used to prepare the inoculum was isolated from the El Colorado farm and identified using the key in Rifai (1969). The Ch-T mixture was incorporated into experimental units chosen at random for the 3x3x2 factorial design (three crops x three levels of solarization x with or without incorporation of chicken manure-*Trichoderma*). Thus, the 18 treatment combinations were: cropping and incorporation of onion (IO = control); solarization for 40 days (S40); solarization for 90 days (S90); incorporation of chicken-*Trichoderma* (ChT); S40×ChT; S90×ChT; cropping and incorporation of broccoli (IB); IB×S40; IB×S90; IB×ChT; IB×S40×ChT; IB×S90×ChT; cropping and incorporation of carrot (ICt); ICt×S40; ICt×S90; ICt×ChT; ICt×S40×ChT; and ICt×S90×ChT. There were three replications per treatment and each repetition had three rows of 6 m long.

Density and viability of sclerotia were determined in each experimental unit; for this, soil samples were taken from the central row 60 days after planting the garlic and at the end of its cycle (March, 2003). The last samples were collected before the removal of the garlic plants. The soil sampling and extraction, counting and determination of sclerotia viability were done as already described. Disease incidence was recorded every 7 days until harvest (March 12, 2003) in the central row of each repetition, considering only the plants located in the central 4 m of the row. The rate of disease increase was estimated [standardized to Weibull's model (b^{-1})], as was the area under the disease progress curve (AUDPC), by the trapezoidal integration method (Campbell and Madden, 1990). Garlic yield was evaluated in the central 4 m of each plot. The reduction or the increase (%) in the number and viability of sclerotia were calculated for each plot in each phase of the study.

Data analysis. All data were subjected to analysis of variance to detect the influence of the main effects (crops and their incorporation, solarization, and incorporation of chicken manure-*Trichoderma*) and their interactions (Martinez-Garza, 1988). Unless otherwise indicated, when significant differences were detected by analysis of variance, Tukey´s test was applied (P = 0.05).

For all analyses the SAS program was used (SAS, 1995). All the variables, except yield, were transformed to ranks with the program PROC RANK (SAS, 1995).

RESULTS

Sclerotium density and viability. In the first phase of the study, because of the high initial density of sclerotia (0.40 sclerotia g^{-1} of soil), a high incidence of white rot was expected where onion was cropped; however, only a low incidence was observed, possibly due to low sclerotia viability (below 40%). Cropping of onion, broccoli or carrot had no significant effect on the density of sclerotia at the completion of this crop cycle (DEC = 0.37 to 0.44 sclerotia g^{-1} of soil, Table 1): the density of sclerotia was similar to the initial density (ID = 0.40 to 0.45 sclerotia g^{-1} of soil). The viability of sclerotia at the beginning of the first phase was between 36 and 38%; by the end of this phase, the only significant reduction was after broccoli (down from 36% to 20%, Table 2, First phase). No significant differences in % reduction of sclerotium viability (%RVEC) and % reduction of inoculum density (%RDEC) were detected (P = 0.05); however, planting broccoli showed a clear tendency to reduce both the number and viability of sclerotia compared with onion (control) (Table 2, First phase).

Cropping and broccoli incorporation significantly (Tukey, P = 0.05) reduced sclerotium number by the end of solarization in the second phase (%RDES), as compared to onion and carrot, but did not affect viability (%RVES) (Tables 1 and 2, Second phase). However, even though the incorporation of crops did not significantly affect sclerotium viability (VES), lower viability was found following the incorporation of broccoli (Table 2, Second phase). At the end of solarization, the number of sclerotia and their viability had diminished significantly (DES: 47 and 70% and VES: 46 and 81%, for 40- and 90-day treatments, respectively), in comparison with non-solarized plots (Table 2, Second phase). The interaction IC×S (Table 1) only affected the density of sclerotia (DES).

Daily maximum temperatures at 15 cm depth varied from 30.7 to 38.5°C and from 30.7 to 39.8°C in treatments receiving 40 (S40) and 90 days of solarization (S90), respectively; in non-solarized soil (NS), the daily maximum temperatures during the 90 days varied between 23.2 and 25.5°C. In solarized treatments, temperatures reached 30.7 to 34.5°C for an average of 10 and 13 h a day (S40 and S90, respectively), and temperatures exceeded 35°C for a maximum of 4 h a day.

In the third phase of the study, 60 days after planting of garlic, the planting and incorporation of crops, and solarization significantly affected *S. cepivorum* levels

Table 1. Analysis of variance of the effect of crop (onion, broccoli and carrot) planting and their incorporation (IC) and solarization (S) on density and viability of *S. cepivorum* sclerotia at the beginning and end of the first phase (December 1, 2001 - April 2, 2002); and at the end of the second phase (August 2, 2002).

Source of variation							
First phase	df	IID	IV	DEC	VEC	%RDEC	%RVEC
Treatements	2	2.67	1.72	1.69	5.2xx	3.2x	1.52
Error	51						
Total	53						
CV		55.3	54.1	56.3	50.5	54.7	56.2
Second phase				DES	VES	%RDES	%RVES
Crops (IC)	1			1.9	3.2*	3.8*	1
Solarization (S)	1			151.6**	86.4**	42.0**	4.8**
ICxS	1			3.9**	2.1	1.5	0.22
Error	50						
Total	53						
CV				21.5	27.2	31.3	48.6

IID: Initial Inoculum Density g^{-1} of soil; IV: % Initial Viability; DEC: Inoculum Density g^{-1} of soil at the end of the crop cycle; VEC: % inoculum viability at the end of the crop cycle; %RDEC: % reduction of inoculum density by the effect of the planted crops; %RVEC: % reduction of inoculum viability by the effect of the planted crops; DES: Inoculum density g^{-1} of soil at the end of solarization for 90 days; VES: % inoculum viability at the end of solarization for 90 days; %RDES: % reduction of inoculum density at the end of solarization for 90 days; %RVES: % reduction of inoculum viability at the end of solarization by 90 days. df: Degrees of freedom ; CV: coefficient of variation.

* Significant at P = 0.05; ** Significant at P = 0.01.

Table 2. Impact of planting and incorporation of onion (IO), broccoli (IB) or carrot (ICt) on density and viability of *S. cepivorum* sclerotia at the beginning and the end of the crop cycle (First phase: December 01, 2001 - April 2, 2002) and at the end of the second phase (August 2, 2002).

Treatments						
First phase	IID	IV	DEC	VEC	%RDEC	%RVEC
Onion (IO)	0.40 a	38.3 a	0.37 a	23.4 a	7.2 a	38.8 a
Broccoli (IB)	0.45 a	35.9 a	0.39 a	20.2 b	11.0 a	43.5 a
Carrot (ICt)	0.44 a	38.3 a	0.44 a	23.4 a	7.3 a	38.6 a
Second phase			DES	VES	%RDES	%RVES
Onion (IO)			0.18 a	13.8 a	52.1 ab	80.4 a
Broccoli (IB)			0.17 a	11.7 a	57.8 a	70.5 a
Carrot (ICt)			0.23 a	15.2 a	43.3 b	66.7 a
Non-solarized (NS)			0.32 a	23.4 a	25.8 c	82.3 a
S40 days			0.17 b	12.5 b	53.0 b	50.8 b
S90 days			0.09 c	4.5 c	74.7 a	84.4 a

IID: Initial inoculum density g^{-1} of soil; IV: % initial viability; DEC: Inoculum density g^{-1} of soil at the end of the crop cycle; VEC: % inoculum viability at the end of the crop cycle; %RDEC: % reduction of inoculum density by the effect of the planted crops; %RVEC: % reduction of inoculum viability by the effect of the planted crops; DES: Inoculum density g^{-1} of soil at the end of solarization for 90 days; VES: % inoculum viability at the end of solarization for 90 days; %RDES: % reduction of inoculum density at the end of solarization for 90 days; %RVES: % reduction of inoculum viability at the end of solarization for 90 days. Means followed by the same letter are not significantly different (Tukey, P = 0.05).

(both density, D60 and % reduction, %RD60) (Table 3); broccoli and solarization had the highest impact on the reduction of both variables (Table 4). However, sclerotium viability (V60 and %IV60) and inoculum density at harvest (FD) were reduced significantly only by solarization. Also, the increase in sclerotium percentage viability (%IV60) was significantly smaller compared with the non-solarized treatments. The viability of sclerotia at the end of the study (FV) was significantly affected by crop incorporation and solarization; the highest FV corresponded to the non-solarized treatments (59.6%) (Table 4).

Chicken manure-*Trichoderma* (Ch-T) incorporation had a significant effect only on %RD60 and the interaction IC×S only affected the variable D60 (Table 3).

Disease incidence. White rot appeared about 120 days after planting the garlic and its incidence was first estimated 14 days later. Initially, disease symptoms were observed in all treatments except S40×ChT, IB×S40, IB×S40×ChT and IB×S90. The disease increased slowly in treatments that received solarization (data not shown). In general, all treatments, except ICt and ICt×ChT, showed a smaller disease incidence with respect to the control (IO).

Solarization for 40 and 90 days and incorporation of broccoli and onion significantly reduced disease incidence (I) and the AUDPC (Table 4). The rate of disease increase (b^{-1}) was significantly reduced only by those treatments involving solarization; both periods of solarization (S40 and S90) had a similar effect, causing reductions of 74 to 84% as compared to non-solarized treatments.

Garlic yield. With the exception of ICt (2.875 t ha^{-1}) and ICt×ChT (3.875 t ha^{-1}), all treatments exceeded the yield of the control (5.875 t ha^{-1}) by 9 to 160 %. In the treatments with solarization (means: S40, 11.625; S90, 13.875 t ha^{-1}) or in combination with broccoli (IB), yields were increased by from 98 to 152%, with respect to the control (IO) (Fig. 1). Incorporation of broccoli (IB) produced a yield (12.712 t ha^{-1}), significantly greater (P = 0.05), by 15 and 24%, than yields after incorporation of onion and carrot, respectively. There was no significant difference in yield between the two solarization periods (S40 and S90) but, in comparison with the non-solarized treatments, they gave increases of 55 and 57%, respectively (Table 4).

DISCUSSION

The low incidence of white rot in onion in the first phase of the study could be explained partly by the absence of species of *Allium* for two consecutive years and the application of allyl disulphide, which would have stimulated the germination of sclerotia in the absence of a host, and caused them to die by starvation or antago-

Table 3. Analysis of variance of the effect of crop incorporation (IC), solarization (S) and soil incorporation of chicken manure-*Trichoderma* (ChT) on density and viability of *S. cepivorum* sclerotia, incidence and progress of disease, and garlic yield in the third phase of the study (September 2, 2002 - March 3, 2003).

Source of variation	df	D60	%RD60	V60	%RV60	FD	FV	I	b^{-1}	AUDPC	YIELD[a]
Incorporation of crops (IC)	2	4.22 *	5.75**	2.6	0.30	0.05	4.83 **	4.93 **	1.9	3.6*	30.2 **
Solarization (S)	2	74.3 **	14.9 **	17.1 **	3.60 x	28.9 **	25.7 **	92.7 **	54.2 **	77.9 **	240.1**
IC x S	4	3.2 *	2.1	1.24	0.32	1.13	0.84	0.6	1.0	1.50	3.83 **
Chicken manure-*Trichoderma* (ChT)	1	0.01	4.56 *	0.50	1.22	0.02	0.05	1.2	0.8	0.43	3.77
IC x ChT	2	0.64	0.60	0.30	0.24	0.62	2.2	0.3	0.7	0.94	0.38
S x ChT	2	2.7	1.93	0.06	0.22	0.44	1.03	2.9	0.2	3.80 *	0.20
IC x S x ChT	4	1.86	1.03	0.34	0.50	0.55	1.41	1.1	0.4	1.21	0.23
Error	36										
Total	53										
CV		27.9	41.7	45.3	58.8	41	50.3	39.2	54.6	43.9	46.5

[a] Garlic yield t ha^{-1} ; D60: Inoculum density g^{-1} of soil evaluated 60 days after planting garlic; %RD60: % reduction of inoculum density between end of solarization and 60 days after planting garlic; V60: % inoculum viability 60 days after planting garlic; %IV60: % increase of inoculum viability between end of solarization and 60 days after planting garlic; FD: Inoculum density at garlic harvest; FV: % inoculum viability at garlic harvest; I: Disease incidence; b^{-1}: Rate of disease increase; AUDPC: Area Under Disease Progress Curve; df: Degrees of freedom ; CV: coefficient of variation.

[*] Significant at P = 0.05; **Significant at P = 0.01.

Table 4. Impact of treatments on density and viability of *S. cepivorum* sclerotia, incidence and progress of disease, and garlic yield in the third phase of the study (September 2, 2002 – March 3, 2003).

Treatments	D60	%RD60	V60	%IV60	FD	FV	I	b^{-1}	AUDPC	YIELD[a]
Onion Incorporation (IO)	0.10 ab	49.4 b	33.1 a	114.8 a	0.11 a	33.5 b	19.4 b	0.006 a	0.420 b	10.767 b
Broccoli Incorporation (IB)	0.08 b	65.7 a	33.1 a	121.1 a	0.11 a	46.5 a	19.1 b	0.007 a	0.390 b	12.712 a
Carrot Incorporation (ICt)	0.14 a	46.1 b	45.9 a	155.6 a	0.12 a	48.0 a	29.9 a	0.01 a	0.750 a	9.610 c
Non-solarized (NS)	0.22 a	42.5 c	60.1 a	164.6 a	0.18 a	59.6 a	51.5 a	0.019 a	1.270 a	5.495 b
Solarized 40d (S40)	0.05 b	67.0 a	33.3 b	130.6 b	0.10 b	45.4 b	10.6 b	0.005 b	0.189 b	13.295 a
Solarized 90d (S90)	0.05 b	51.6 b	18.8 b	96.3 c	0.06 c	22.9 c	6.3 c	0.003 b	0.114 c	13.850 a

[a] Garlic yield t ha^{-1}; D60: Inoculum density g^{-1} of soil evaluated 60 days after planting garlic; %RD60: % reduction of inoculum density between end of solarization and 60 days after planting garlic; V60: % inoculum viability 60 days after planting garlic; %IV60: % increase of inoculum viability between end of solarization and 60 days after planting garlic; FD: Inoculum density at garlic harvest; FV: % inoculum viability at garlic harvest; I: Disease incidence; b^{-1}: Rate of disease increase; AUDPC: Area Under Disease Progress Curve; Means followed by the same letter are not significantly different (Tukey, P = .05)

nism (Coley-Smith, 1990). It may also have been because the sclerotia showed a relatively low viability (under 40%). In addition, the delayed planting of the crops (December) would not have favored the development of the disease. In Brazil, the incidence of white rot in garlic planted in the summer varied between 0 and 35%, whereas in autumn plantings incidence was from 68 to 88% (Pinto *et al.*, 1998). These authors suggest that disease incidence will depend both on the state of development of the host and on the suitability of soil conditions (mainly temperature) for the development of the pathogen and the host root system and, therefore, the production of exudates. Natural decline of the viability and numbers of *S. cepivorum* sclerotia have been attributed to unfavorable temperatures and to sclerotium germination under the influence of humidity (Adams, 1987), which combined with the absence of an *Allium* host, will lead to inoculum reduction by starvation or destruction of the propagules by soil microorganisms (Papavizas and Lumsden, 1980; Rahe and Utkhede, 1985).

At the end of the second phase (i.e. at the time of garlic planting), the sclerotial inoculum was reduced by 52% (%RDES) and sclerotium viability by 80% (%RVES) where onion had been incorporated (Table 2). However, the remaining 20% viable sclerotia (i.e. 0.036 sclerotia g^{-1} of soil) were sufficient to cause a 45% incidence of white rot and a 61% reduction in garlic yield with respect to the best treatment (IB×S90×ChT). Delgadillo *et al.* (2004) found that, with a natural reduction of 54% in the initial inoculum density (0.09 sclerotia g^{-1} of soil was reduced to 0.04 in a period of 1 year), the incidence of garlic white rot was 38%, causing a yield reduction of 62% with respect to the best treatment.

Although broccoli, onion and carrot had similar effects on inoculum density and on the reduction of % viability (%RVEC varied between 39 and 44%) at the end of their cycle, only broccoli significantly reduced sclerotial viability in the first phase (Table 2). The root exudates released during broccoli development might have stimulated sclerotium germination and/or been toxic to them, which, along with other factors such as those mentioned previously, would have contributed to the reduction of the inoculum. The benefits of broccoli with respect to carrot and onion were more evident 3 months after their incorporation (Table 2, Second phase), when a greater reduction in the inoculum density was observed.

It is known that cruciferous plants contain thioglucosides, which, during tissue decomposition, give rise to isothiocyanates, sulfides of various types (including diallyl and propyl allyl) mercaptans, and nitriles, among others (Lewis and Papavizas, 1971); such compounds have fungistatic and/or fungicidal properties (Lewis and Papavizas, 1971; Gamliel and Stapleton, 1993a; Angus *et al.*, 1994). It is known that volatiles released during decomposition of cruciferous tissues stimulate or inhibit the germination of sclerotia of *S. cepivorum*, depending on the amount of tissue incorporated into the soil (Zavaleta-Mejía *et al.*, 1992a). The incorporation of crucifers is reported to reduce the incidence and severity of white rot and the number of sclerotia (Villar *et al.*, 1990; Zavaleta-Mejía *et al.*, 1992b). In the present study, the density and viability of the inoculum of treatment IB recorded before planting garlic led to a 49% incidence of white rot (similar to the onion incorporation treatment); however, this incidence was 34% lower than that of treatment ICt (I = 74%), and the yield in IB was 16 and 25% higher (Table 4) than in the treatments incorporating onion and carrot, respectively.

Planting and incorporation of carrot did not significantly affect either inoculum density or viability and resulted in the greatest incidence of white rot (74%). These results contrast with those of Banks and Edgington (1989), who found a significant reduction in the level of inoculum (from 0.45 to 0.17 sclerotia g^{-1} of soil) with carrot planting.

Solarization, for 40 and 90 days, alone or combined with other treatments, was the strategy that gave the best results, confirming previous reports (Pullman *et al.*, 1981; Zavaleta-Mejía *et al.*, 1992b; Zavaleta-Mejía *et al.*, 1994; Delgadillo *et al.*, 2004). In our study, temperatures reached 30.7 to 39.8°C at 15 cm depth in solarized soil, which fall within the range of sub-lethal temperatures (Pullman *et al.*, 1981; Tjamos and Fravel, 1995); these temperatures were relatively low compared to those reported in other solarization studies to control several soil-borne plant pathogens (Gamliel y Stapleton, 1993b; Pirketon *et al.*, 2000); however most likely soil temperatures at 0 to 10 cm depth could have been near or above 40°C. The length of time for which sclerotia remained exposed to those temperatures during the solarization (a daily average of 10-13 h with temperatures from 30.7 to 34.5°C and a daily maximum of 4 h with temperatures ≥35°C) might have affected their germination, survival and pathogenicity. It has been reported that viability of *S. cepivorum* sclerotia is considerably reduced by exposure to temperatures above 30°C (Crowe and Hall, 1980) and exposures to 40°C for 39 h killed at least 50% of them (Adams, 1987).

Entwistle and Munasinge (1990) found that *S. cepivorum* exposed to sublethal temperatures, 35 or 40°C for 3 to 7 or 24 to 48 h, respectively, were colonized by bacteria and fungi, mycelium production in agar was delayed and the colonies were smaller compared with unexposed sclerotia; survival and germination in soil were also reduced. Also, Tjamos and Fravel (1995) found that exposure of microsclerotia of *Verticillium dahliae* for 10 to 14 h at temperatures from 31 to 38°C delayed their melanization and germination. The increase in microbial processes induced by solarization (Katan, 1981) could affect *S. cepivorum* by increasing its vulnerability to soil microorganisms; Beuchat (1984) and Russel

(1984) have indicated that the exposure of propagules of soil borne-plant pathogens to sub-lethal temperatures and other stress factors could increase exudation of nutrients favouring invasion by the soil microflora. All these effects reduce the survival, mycelia growth and the pathogen capacity to infect the host (Katan *et al.*, 1976; Katan, 1981).

In spite of all these injurious effects, some sclerotia survived, germinated and caused 9 and 5.6% incidence of white rot in the S40 and S90 treatments, respectively. Nevertheless, these figures were extremely low compared with treatments IO (control, 45%), IB (49%) and ICt (74%). The rate of disease increase (b^{-1}) was also delayed considerably by solarization (from 74 to 84%), which in turn was reflected in a diminution of the AUDPC (between 84 and 91%), with respect to the non-solarized treatments (Table 4). All this was translated into increases of 98% (S40) and 136% (S90) in garlic yield with respect to the treatment IO (Fig. 1).

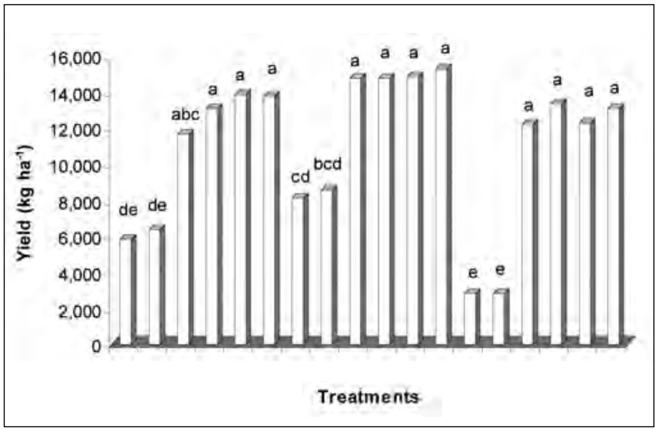

Fig. 1. Effect of planting and incorporation of onion (IO), broccoli (IB) or carrot (ICt) crop residues, solarization for 40 (S40) or 90 (S90) days, and incorporation of chicken manure-*Trichoderma* (ChT) on garlic yield. Bars with the same letter do not differ significantly (Tukey, P = 0.05). Each bar represents the average of three replicates.

The addition of chicken manure-*Trichoderma* (ChT) at the time of garlic planting did not affect sclerotial viability but did affect inoculum density evaluated 60 days after planting, which confirmed previous results obtained in a pot test (Ulacio-Osorio *et al.*, 2004). However, this effect was not reflected in significant disease reduction or in an increase of garlic yield. In contrast, Gamliel and Stapleton (1993b), found that chicken manure applied before or after the solarization was very effective to control root galling caused by *Meloidogyne incognita* in lettuce, as compared with non-solarized treatments; also the number of cfu of *Pythium ultimum* was reduced up to 100% when chicken manure was incorporated to soil before or after solarization and lettuce yield was increased up to 21.7% (in autumn) and 78.7% (in spring), in treatments solarized before the incorporation of chicken manure, as compared to those non-solarized.

The greatest beneficial impact of the combination of solarization with planting and incorporation of crops on garlic yield was the broccoli-solarization combination. These results indicate that solarization combined with the incorporation of crop residues provides an additional advantage; besides improving soil structure, it also increases microbial dynamics and, therefore, increases the beneficial microorganisms that can antagonize the pathogen (Stapleton and Duncan, 1998). Villar *et al.* (1990) found, in greenhouse conditions, that a broccoli-solarization combination gave a greater reduction on onion white rot severity (78 to 83%) than treatment with solarization alone (67 to 79%). In other studies, increases in onion yield with a combination of solarization and incorporation of crucifers have been reported (Zavaleta-Mejía *et al.*, 1992b; Zavaleta-Mejía *et al.*, 1994).

The greater effectiveness of the combination of crucifer incorporation and solarization might be due to the entrapment, by the polyethylene tarp, of the fungitoxic gases given off during decomposition of the cruciferous residues (Ramirez-Villapudua and Munnecke, 1988).

Our results indicate that a break from susceptible cropping and incorporation of broccoli, combined with solarization, was the strategy giving greatest reduction in the density and viability of *S. cepivorum* inoculum, the lowest incidence of white rot and the greatest yield of garlic. However, the magnitude of the economic benefit remains to be determined, as does the ecological one, when combining these practices.

ACKNOWLEDGEMENTS

Financial support was provided by the Universidad Centroccidental Lisandro Alvarado-Venezuela and the Colegio de Postgraduados-México.

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Received May 24, 2005
Accepted March 20, 2006