Effect of Cauliflower Residue Amendments and Soil Solarization on Verticillium Wilt Control in Artichoke

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ABSTRACT

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The effect of fresh cauliflower residue amendment alone and with a low dose of metham sodium (MS) combined with soil solarization was investigated for the control of Verticillium wilt of artichoke in two commercial fields under artichoke–cauliflower rotation. Treatments were a factorial combination of three main plots (an unamended control, soil amended with cauliflower residue, and a combination of cauliflower residue and a low dose of MS) and two subplots (application of a plastic cover or uncovered). Inoculum densities of *Verticillium dahliae* were measured before and after soil treatments as well as disease incidence, symptom severity, and yield. Although soil solarization reduced inoculum of *V. dahliae* and the incidence of Verticillium wilt of artichoke, no added benefit was obtained when solarization was used with cauliflower residue amendments. In addition to toxic volatile compounds, other mechanisms could be involved in disease suppression because the effects of incorporating cauliflower residue were not enhanced by the plastic covering. The effect of cauliflower residues on populations of *V. dahliae* microsclerotia in soil was inconsistent, possibly due to varying pretreatment inoculum levels. Treatments with cauliflower residue amendments and low doses of MS maintained low inoculum densities in the fields until the end of the growing season and significantly reduced the percentage of infected plants.

Additional keywords: biofumigation, crucifer residues, *Cynara cardunculus* L. var. *scolymus* (L.) Fiori, glucosinolates

Artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori) is an important vegetable crop in Spain, where 32% of the total European production is grown (18). In recent years, Verticillium wilt, caused by *Verticillium dahliae* Kleb., has severely affected commercial artichoke production in the Comunidad Valenciana Region located on the eastern-central Mediterranean coast (2). *V. dahliae* is a soilborne pathogen that is distributed worldwide and affects hundreds of herbaceous and woody crops (30). In the management of this disease, it is essential to reduce inoculum levels in soil below critical thresholds before planting (9,39,46).

Although currently available soil fumigants are only slightly effective on many plant-pathogenic fungi (20), metham sodium (MS) is widely used against *V. dahliae* in Spanish artichoke fields because of its relatively low cost and easy application. In moist soil, MS undergoes decomposition to methyl isothiocyanate, the active compound that possesses broad-spectrum

biological activity in soil (26,43). Methyl isothiocyanate is susceptible to enhanced biodegradation, a process that can seriously compromise its efficacy by unusually rapid breakdown in soil (26). Briefly, this phenomenon is caused by adaptation of microbes to use the pesticide as an energy source and may be induced by repeated or even single applications of the pesticide to a field (15,26,44).

The efficacy of control of Verticillium wilt based on crop rotation is limited by the long survival of microsclerotia in soil and lack of host specificity (38). Moreover, *V. dahliae* can persist in symptomless host roots, providing a means of multiplication and dissemination that frequently invalidates crop rotation programs (21,35). Because *Verticillium*-resistant artichoke cultivars are not commercially available, there is a need to incorporate alternative techniques in Verticillium wilt management programs.

The use of heat and generation of anaerobic conditions under plastic has been shown to reduce the survival of many plant pathogens (9,33). Soil solarization has successfully controlled Verticillium wilt in cotton (28), olive (25), fruit trees (37), and eggplant (40). The effectiveness of soil solarization in controlling Verticillium wilt of globe artichokes in Greece has been demonstrated (42).

The potential of secondary plant products released from *Brassica* spp. residues

following their incorporation into soil to suppress pests has long been recognized in a process named “biofumigation” (1,26). Nevertheless, field experiments assessing the effectiveness of *Brassica* spp. crop residues on reducing soilborne pathogens have had inconsistent results ranging from complete to partial suppression to no effects (9,13,26,27,38,39,46). Mechanisms for control have been attributed partially to the chemical breakdown products of glucosinolates (GLS), the characteristic constituents of crucifer crops. GLS hydrolysis releases, among other secondary compounds, isothiocyanates (ITCs) that have either fungistatic or fungicidal properties (4,38,39). Other reasons for disease suppression from crucifer residue incorporation include nonhost or trap crop effects, and indirect effects on the pathogen associated with changes in the populations of antagonistic organisms, as well as effects of compounds released from the tissues that may not be related to GLS or ITCs (26). In previous studies, soil amendments of crucifer crop residues such as cauliflower, broccoli, or cabbage (24,34) combined with solarization produced a greater variety of fungicidal compounds than crucifer residue alone (19).

The objectives of this study were to (i) evaluate the effect of incorporating fresh cauliflower residue alone or with a low dose of MS on *V. dahliae* inoculum density, Verticillium wilt development, and artichoke yield; and (ii) determine if there is an additional effect of soil solarization when combined with these treatments. A preliminary report of this study was presented in the Sixth International Symposium on Artichoke, Cardoon, and Their Wild Relatives (7).

MATERIALS AND METHODS

Treatments and experimental design.

Experiments were conducted in two fields located in Benicarló (Castellón) from June 2004 to May 2005 (field 1) and from June 2005 to May 2006 (field 2). Both fields had a history of severe Verticillium wilt of artichoke and were under artichoke–cauliflower rotation, which is very common in the area of study. To our knowledge, Verticillium wilt has never occurred in commercial cauliflower crops planted in heavily infested soils located in this area. Furthermore, isolates of *V. dahliae* from artichoke were not pathogenic to cauliflower in greenhouse pathogenicity tests

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(M. Berbegal and J. Armengol, *unpublished*). Both fields had a sandy clay loam texture with a pH of 8.0 and 1.6% organic matter.

The experimental design was a three-by-two factorial combination of three soil treatments and two plastic-cover treatments arranged in a split-plot design with four replications. Main plots consisted of an unamended control, incorporation of cauliflower residue, and a combination of cauliflower residue and a low dose of MS treatment (350 liters a.i. ha⁻¹). Cauliflower plants (cv. Marine) were completely removed from the field in unamended plots and fresh residues incorporated after harvest only in the amended ones. The subplots consisted of the application of a plastic cover or uncovered. In both fields, cauliflower residue was homogeneously disk-incorporated twice to a depth of 25 to 30 cm on June 14 and 15 in fields 1 and 2, respectively.

In field 1, each subplot was 13 m long and seven raised beds wide (1 m between bed centers with an in-row spacing of 0.8 m). An average of 140 kg of fresh cauliflower residue after cauliflower harvest remained per amended subplot and was incorporated (2 kg of wet biomass per square meter). In field 2, each subplot was 7 m long and seven raised beds wide (1 m between bed centres with an in-row spacing of 0.8 m). An average of 100 kg of fresh cauliflower residue per amended subplot was incorporated (2.4 kg of wet biomass per square meter).

On 17 June in both experiments, fields were irrigated and MS applied. Thereafter, the corresponding subplots were covered with transparent 50- μ m-thick PE plastic sheets. The edges were sealed with soil. After 35 days, plastics were removed and beds were reshaped. One week later, stumps of artichoke cv. Blanca de Tudela free from the pathogen were planted. Field preparation in both experiments consisted of cultivation practices common to commercial artichoke production in this region.

In both fields, soil temperatures were continuously recorded with a data logger (WatchDog data logger, model 450; Spectrum Technologies Inc., Plainfield, IL) at depths of 10, 20, and 30 cm in solarized and nonsolarized subplots.

***V. dahliae* inoculum density assay.** Pretreatment soil samples were collected in each field at the beginning of June. Additionally, three post-treatment soil samples were taken immediately after plastic removal (35 days after treatments were applied); in October, 2 months after artichokes were planted (119 days after treatments were applied); and in May, at the end of the growing season (336 days after treatments were applied). Each subplot had three sampling areas marked by a "V" pattern that included nine artichoke plants each for soil sampling and disease assessment.

Four soil cores were collected to a depth of 10 cm from each sampling area and bulked into a single soil sample per subplot. Samples were air dried for 4 weeks, treated with DL-methionine to break microsclerotia dormancy, and assayed for *V. dahliae* inoculum density using the modified Anderson sampler technique (10) and NP-10 medium (23) as previously described (8). Plates were incubated in the dark at 23°C. After 3 weeks, plates were washed with a stream of water to remove soil particles and aerial mycelia in order to facilitate the identification of colonies of *V. dahliae* under the stereomicroscope. Colonies of *V. dahliae* were identified and counted based on the morphology of microsclerotia formed in the agar, their distribution within the medium, and the absence of dark hyphae or dark mycelium (20). The inoculum density (ID) in a sample was estimated as the number of microsclerotia per gram of dry soil (ms g⁻¹).

Disease assessment and yield. Severity of plant symptoms (SS) was recorded in four artichoke plants randomly selected in each sampling area in October, 2 months after planting. October was the best time for the assessment because, from November until the end of the growing season, the continuous harvest of artichoke heads damages the plants, confounding evaluation of symptoms. A 0-to-5 rating scale was used (11), taking into account foliar symptoms (yellowing and wilting) and plant stunting, where 0 = no symptoms, 1 = 1 to 9% of affected leaf surface and very slight stunting, 2 = 10 to 24% of affected leaf surface and very slight to moderate stunting, 3 = 25 to 50% of affected leaf surface and very slight to severe stunting, 4 = more than 50% of affected leaf surface and very slight to severe stunting, and 5 = dead plant. Mean values of SS corresponding to each subplot were obtained for the data analyses.

To assess disease incidence in May at the end of the growing season (336 days after treatments were applied), four basal leaves were taken from four random plants in each of the three sampling areas marked per subplot. In the laboratory, the sampled leaves were washed with tap water, surface sterilized for 1 min in a sodium hypochlorite solution (1.5% active chlorine), and rinsed twice with distilled sterile water. Subsequently, four small internal fragments of vascular tissue from each leaf were plated onto potato dextrose agar amended with streptomycin sulfate at 0.5 mg ml⁻¹ (PDAS) and incubated at 25°C. The colonies that developed on the plates were examined periodically and *V. dahliae* was identified based on the presence of microsclerotia and conidiophore morphology. Disease incidence (DI) was rated in each subplot based on the percentage of infected plants (confirmed by lab isolation). Yields in each subplot were deter-

mined by the total weight (g) of commercial bud heads per plant recorded during all the cropping season.

Data analyses. Each treatment mean (ID, DI, SS, and yield) was calculated from the corresponding values in each subplot over the 2 years of the experiment. Percentage data were transformed into arcsine ($DI/100$)^{1/2} before analysis. Data on ID, DI, and yield were analyzed using GLM procedure of SAS (version 9.0; SAS Institute Inc., Cary, NC) according to the experimental design in split-plot, including main effect terms for block, amendment, covering, and terms for the interaction amendment-covering. SS data were transformed using the rank procedure of SAS before GLM analysis. Means were compared by the least significant difference test ($P < 0.05$).

RESULTS

Soil temperatures. Mean soil temperatures recorded during both years in the upper 30 cm of soil were about 5°C higher in solarized subplots than in the nonsolarized subplots. Maximum soil temperatures in solarized subplots in field 1 were 40.2, 34.5, and 31.6°C at 10, 20, and 30 cm, respectively. In field 2, maximum temperatures in solarized subplots were 38.4, 33.5, and 31.0°C at 10, 20, and 30 cm, respectively.

***V. dahliae* inoculum density.** *Field 1.* Pretreatment inoculum densities were similar for all treatments (5 to 6 ms g⁻¹ of soil) except in the unamended control and the unamended plastic covered, which had a mean ID of 12 and 11 ms g⁻¹ of soil, respectively (Fig. 1). Thirty-five days after treatment application, ID significantly decreased ($P = 0.0028$) compared with the unamended control in all treatments but cauliflower amendment alone. The numbers of microsclerotia remained low until the end of the growing season in all treatments except in the unamended control, where ID increased to pretreatment levels. ID remained below 1 ms g⁻¹ of soil only in treatments combining solarization with cauliflower residue incorporation regardless of the application of MS.

Field 2. Pretreatment inoculum densities in field 2 ranged from 10 to 23 ms g⁻¹ (Fig. 1). Thirty-five days after treatment application, ID significantly decreased ($P = 0.0019$) compared with the unamended control only in those treatments where plastic was used, regardless of the application of MS or cauliflower residues. During the growing season, inoculum densities decreased variably depending on the treatments and generally increased again at the end of the growing season, especially in the unamended control (Fig. 1). ID was lower at the end of the growing season compared with pretreatment levels only in subplots treated with MS where cauliflower residue was applied, regardless of the use of plastics (Fig. 1).

Disease incidence and symptom severity. There was a significant effect of soil amendment and plastic covering on mean DI values in both fields (Table 1). Analysis of variance showed no significant amendment-covering interactions for DI, suggesting that the effect of the covering is

independent of the soil amendment treatment (Table 1). In both fields, percentages of infected plants (DI) were significantly lower in treatments with cauliflower residue and MS than in unamended subplots (Table 2). In subplots treated with MS where cauliflower residue was incorpo-

rated, mean DI decreased by 27% in field 1 and 39% in field 2 relative to the unamended control. No significant differences were observed in DI values between treatments with and without cauliflower residue (Table 2). Mean values of DI in solarized subplots were significantly lower

Fig. 1. Inoculum density of *Verticillium dahliae* in the different treatments prior to or at various times after the treatments were applied in fields 1 and 2. The data are the mean of four replications. The vertical bars are the standard errors of the mean. C: cauliflower residue incorporation; CM: cauliflower residue incorporation with metham sodium; plastic: solarization treatment; CM+plastic: cauliflower residue incorporation with metham sodium and solarization.

Table 1. Effects of variables on disease incidence, symptom severity, and yield

Variables	df ^x	Disease incidence (%)		Symptom severity (0 to 5) ^w		Yield (g per plant)	
		MS ^y	<i>P</i> value ^z	MS	<i>P</i> value	MS	<i>P</i> value
Field 1							
Block	3	42.8	0.4686	17.4	0.4408	2,812,741.5	0.0598
A (Amendment)	2	436.2	0.0129	132.8	0.0208	2,273,898.9	0.0543
Block × A (error a)	6	44.5	...	16.8	...	461,748.3	...
B (Covering)	1	6,767.3	0.0001	260.0	0.0005	3,391,520.2	0.0192
A × B	2	159.1	0.1800	69.5	0.0130	1,249,540.5	0.1012
Block × B (A) (error b)	9	76.2	...	9.0	...	418,272.9	...
Field 2							
Block	3	388.5	0.0610	6.0	0.8022	529,298.9	0.1258
A (Amendment)	2	652.0	0.0081	18.0	0.4219	270,412.2	0.3030
Block × A (error a)	6	54.5	...	18.0	...	184,369.8	...
B (Covering)	1	3,297.1	0.0038	486.0	0.0002	5,275,312.7	0.0055
A × B	2	725.0	0.0840	18.0	0.3227	411,980.2	0.3969
Block × B (A) (error b)	9	219.5	...	14.0	...	401,668.3	...

^w Symptom severity was rated using the following scale: 0 = no symptoms, 1 = 1 to 9% of affected leaf surface and very slight stunting, 2 = 10 to 24% of affected leaf surface and very slight to moderate stunting, 3 = 25 to 50% of affected leaf surface and very slight to severe stunting, 4 = more than 50% of affected leaf surface and very slight to severe stunting, and 5 = dead plant.

^x Degrees of freedom.

^y MS = mean square.

^z Significance level $P < 0.05$.

than in nonsolarized plots (Table 2). Mean DI in treatments involving solarization were 62 and 49% lower than nonsolarized in fields 1 and 2, respectively. In treatments without solarization, cauliflower residue incorporation regardless of MS application reduced mean DI by 13% relative to the unamended control in both fields (Fig. 2).

Plastic covering had a significant effect on mean SS in both fields (Table 1). Mean values of SS in solarized subplots were significantly lower than in nonsolarized plots (Table 2). The cauliflower residue amendment significantly reduced the percentage of SS only in field 1 (Table 2). In treatments without solarization, incorporation of cauliflower residue regardless of

MS application resulted in lower SS than in the unamended control in both fields (Fig. 2).

Yield. Solarization had a significant effect on yields in both fields (Table 1). The application of a plastic cover increased yield by 16 and 40 % in fields 1 and 2, respectively, relative to the uncovered plots (Table 2). In treatments without solariza-

Table 2. Effects of soil amendments (cauliflower residue, no cauliflower residue, and cauliflower residue combined with metham sodium [MS] treatment), and plastic (solarization and no solarization) on disease incidence, symptom severity, and yield

Amendments	Disease incidence (%)		Symptom severity (0 to 5) ^w		Yield (g per plant)	
	Field 1	Field 2	Field 1	Field 2	Field 1	Field 2
No cauliflower residue	62.4 a ^{x,y}	62.8 a	2.7 a ^z	3.7 a	4,431.8 a ^y	2,751.5 a
Cauliflower residue	63.5 a	52.3 b	2.0 b	3.6 a	5,496.9 b	2,997.3 a
Cauliflower residue + MS	45.7 b	38.5 b	1.9 b	3.5 a	4,921.4 ab	2,637.5 a
Covering						
No solarization	82.8 a	67.8 a	2.6 a	4.0 a	4,574.1 a	2,326.6 a
Solarization	31.7 b	34.6 b	1.8 b	3.2 b	5,325.9 b	3,264.3 b

^w Symptom severity was rated using the following scale: 0 = no symptoms, 1 = 1 to 9% of affected leaf surface and very slight stunting, 2 = 10 to 24% of affected leaf surface and very slight to moderate stunting, 3 = 25 to 50% of affected leaf surface and very slight to severe stunting, 4 = more than 50% of affected leaf surface and very slight to severe stunting, and 5 = dead plant.

^x Mean of four replications (12 artichoke plants in each replication).

^y GLM. Values followed by the same letters within a column are not significantly different according to the least significant difference test ($P < 0.05$).

^z RANK+GLM. Values followed by the same letters within a column are not significantly different according to the least significant difference test ($P < 0.05$).

Fig. 2. Disease incidence (%), symptom severity, and yield (g per plant) in the different treatments in fields 1 and 2. Symptom severity was rated in a scale of 0 to 5, where 0 = no symptoms, 1 = 1 to 9% of affected leaf surface and very slight stunting, 2 = 10 to 24% of affected leaf surface and very slight to moderate stunting, 3 = 25 to 50% of affected leaf surface and very slight to severe stunting, 4 = more than 50% of affected leaf surface and very slight to severe stunting, and 5 = dead plant. Values are the mean of four replications and vertical bars are the standard errors of the mean.

tion, cauliflower residue alone increased yield by 45% relative to the unamended control in Field 1 (Fig. 2).

DISCUSSION

Soil solarization was highly effective in reducing the inoculum density of *V. dahliae*. Maximum soil temperatures recorded were in the range of temperatures necessary to eradicate or reduce inoculum of *V. dahliae* in soil according to values reported by other authors (32,41). Covering the soil with transparent PE plastic for only 1 month in summer significantly reduced the incidence of Verticillium wilt in artichoke and increased the yield compared with the uncovered subplots. Our results agree with those of Tjamos and Paplomatas (42), who demonstrated that soil solarization successfully controlled Verticillium wilt of artichoke in Greece as well as increased yields.

Results from different studies have demonstrated the effectiveness of crucifer residue incorporation in the reduction of both *V. dahliae* microsclerotia in soil and the incidence of Verticillium wilt in cauliflower (39,46) and potato (13). Therefore, biofumigation alone or with low doses of MS was evaluated in commercial fields as an alternative to control Verticillium wilt of artichoke. However, in this experiment, the effects of cauliflower amendments alone and in combination with MS cannot be compared as accurately as the effects of solarization. The effect of the various treatments on reducing the ID relative to the control was inconsistent. The results suggest that the effectiveness and the magnitude of the reduction in the field might depend on pretreatment inoculum levels. ID in treatments with cauliflower residues alone or in combination with soil solarization remained low at the end of the growing season in field 1, where the initial population density recorded was relatively low. In the untreated control, ID increased to pretreatment levels by the end of the season in both fields. This could be explained partially by the release of microsclerotia from infected artichoke debris incorporated into the soil (8,17,25).

Following an initial decline in microsclerotia numbers in soil treated with MS, populations have been reported to either increase or return to preplanting levels toward the end of the season (39). In our study, the effect of cauliflower incorporation combined with low doses of MS resulted in an ID which was maintained all season and significantly reduced the number of infected plants. However, we cannot determine if this effect was due to the combination of biofumigation and MS treatment or MS alone.

Solarization, when combined with the incorporation of cauliflower residue, provided no added benefits in terms of maintaining low ID at the end of the growing season. Our results are in accordance with

the findings of Subbarao et al. (39) and Pinkerton et al. (31), who found no evidence of synergistic interactions between solarization and crucifer residue incorporation to control Verticillium wilt. In contrast, Blok et al. (9) demonstrated the potential of the combination of organic amendments and tarping to control *V. dahliae* and other persistent soilborne pathogens. Inconsistent results may reflect the varying conditions under which the experiments are conducted (45), including the quality or composition of the plant material or differences in the amount and preparation of tissue incorporated (9,12–14,22,27,29).

According to previous studies, the strategy of covering the field with plastics to retain volatile compounds in soil released by crucifer crop residues is known to increase their effect on microsclerotia of *V. dahliae* and to increase MS efficacy to control the disease (5,6,9,16,19,36). Mechanisms of disease control when crucifer amendments are incorporated into soil are varied and often unknown; however, it has been widely assumed that they are based on the release of toxic volatile compounds (14,29,45). These mechanisms were not studied specifically in our experiments; however, results suggest that factors in addition to volatile compounds could be involved because their effects were not enhanced when retained in soil by plastic covering.

Reductions of ID were not associated with significantly lowered DI in all the treatments as reported by other authors (9,29,31). These authors concluded that the reduction of ID is not a necessary condition for reducing wilt severity because other specific suppressive mechanisms could be involved. Nevertheless, Tjamos and Paplomatas (42) observed that reductions in numbers of microsclerotia of *V. dahliae* in soil were positively correlated with significant reductions in the percentage of diseased artichoke plants as well as yield increases. High percentages of disease incidence associated with low inoculum densities of *V. dahliae* in soil even in untreated control subplots were observed during the growing season. This phenomenon has been reported for the incidence of Verticillium wilt in artichoke (8), cotton (3), and olive (25). ID of the pathogen in the untreated control subplots in our experiments showed strong temporal variation, which has been observed in fallow soils (28) and olive tree plantations (25).

Additionally, no positive correlation was observed between DI and SS, which was previously reported in artichoke fields (8). In one of the fields in this experiment, the incorporation of cauliflower residue significantly increased total weights of commercial buds per plant. In other studies, *Brassica* spp. residues, when incorporated to control Verticillium wilt in cauliflower, provided total yields comparable to soil

chemical treatments (39) and enhanced growth of cauliflower plants by a combination of increased root growth and nutrient uptake (38).

Soil solarization effectively managed Verticillium wilt of artichoke and would be compatible with normal agronomic practices developed for artichoke production. Rotation of artichoke with cauliflower for crop residue incorporation with or without low doses of MS could be used to manage Verticillium wilt in fields with low preplanting ID. Growers could easily incorporate cauliflower residue after harvest for *V. dahliae* microsclerotia reduction in soil. Further research is needed to determine whether larger amount of *Brassica* residues as well as better disruption or quality of tissue incorporated to the field would improve the effects of cauliflower mediated disease suppression in highly infested fields.

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