Combined Soil Treatments and Sequence of Application in Improving the Control of Soilborne Pathogens

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ABSTRACT

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The effects of reduced doses of methyl bromide (MB) or metham sodium, heating, short solarization, and soil microbial activity, alone or in combination, on survival of soilborne fungal pathogens were tested in a controlled-environment system and field plots. Sublethal doses of heating or MB delayed germination of *Sclerotium rolfsii* sclerotia. Combining MB and heating treatments was more effective than either treatment alone in controlling *S. rolfsii* and *Fusarium oxysporum* f. sp. *basilici*. The application heating followed by fumigation with MB, was significantly more effective in delaying and reducing germination of *S. rolfsii* sclerotia and in controlling *F. oxysporum* f. sp. *basilici* than the opposite sequence.

Further, incubation in soil and exposure to microbial activity of previously heated or MB-treated sclerotia increased the mortality rate, indicating a weakening effect. Similarly, incubation of chlamydospores of *F. oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *radicis-lycopersici* in soil in the field after fumigation further reduced their survival, confirming the laboratory results. In field tests, combining MB or metham sodium at reduced doses with short solarization was more effective in controlling fungal pathogens than either treatment alone. Treatment sequence significantly affected pathogen control in the field, similar to its effect under controlled conditions. This study demonstrates a frequent synergistic effect of combining soil treatments and its potential for improving pathogen control and reducing pesticide dose, especially when an appropriate sequence was followed.

Additional keywords: biocontrol, fumigant, soil disinfestation, vapam.

The coming phaseout of methyl bromide (MB), a major soil fumigant worldwide, has intensified the need for new control methods, as well as the improvement of existing ones, to achieve acceptable levels of control without negatively affecting the environment. Combining tactics is a major tool in integrated pest management and is one of the most feasible approaches in this regard (17). Indeed, the combination of fumigants at reduced doses, soil solarization, antagonists, or other agents improves pathogen control (3,9,10,13,14,16,21-23). Although MB will be phased out in developed countries in 2005, its use will continue for a specified period in developing countries, as well as for particular critical uses in other countries. Therefore, means of reducing MB usage, while maintaining its effectiveness, are still needed, and this holds true for other fumigants as well. The use of sublethal doses of killing agents weakens the pathogen and enables effective control while minimizing dose (10,11,18,20,23). Thus, weakening pathogen propagules by sublethal soil treatment increases their vulnerability to other agents in the soil, such as chemicals, heat, and soil microbial antagonists. This can lead to the enhancement of biological control of the partially damaged propagules by soil organisms (2,10,18,20).

The objectives of this study were to test the possibility of reducing soil fumigant doses and shortening soil solarization periods by successive treatment applications in two application

sequences in a controlled system and in soil under field conditions.

MATERIALS AND METHODS

Soils. Two types of soil were used: an agricultural sandy soil from Rehovot, Israel (0.5% organic matter, 95.7% sand, 3.8% clay, pH 7.9, water holding capacity 9%, wt/wt), and an acid-washed marine sand (Sigma Chemical Company, St. Louis) with a water-holding capacity of 5% (wt/wt), which was sterilized by autoclaving (120 °C for 20 min).

Pathogens and inoculum preparation. Propagules of four pathogenic soil fungi were used in these studies. Macroconidia of *Fusarium oxysporum* Schlechtend. f. sp. *basilici* (Dzidzarya) Armstr. & Armstr. were collected from sporulating lesions on stems of sweet basil (*Ocimum basilicum* L.) from a crop severely affected by the pathogen. The outer tissue of stems showing pink spore masses was stripped off with a scalpel and kept at room temperature until use. Immediately before use, the peel was cut into 1 cm lengths.

Soil from a greenhouse with diseased basil plants naturally infested with *F. oxysporum* f. sp. *basilici* and soil from a field with diseased tomato plants infected with *F. oxysporum* f. sp. *radicis-lycopersici* were collected and air-dried and served as natural inocula. The soils were stored for up to 9 months at room temperature, and before use, the density of chlamydospores as determined by microscopic observation, was assayed by a soil dilution method (12). Samples of *F. oxysporum* isolates from these soils were used to inoculate their respective host (basil or tomato) in order to verify their identity.

Chlamydospores of *F. oxysporum* f. sp. *melonis*, originally isolated from diseased muskmelon plants, were produced in soil.

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Conidia of the pathogen were suspended in water, mixed with sandy Rehovot soil and incubated for 100 to 120 days at 27°C, after which only chlamydospores remained in the soil, as verified by microscopic examination. The infested soil was air-dried and stored at 8°C until use.

Sclerotium rolfsii Sacc. was initially isolated from diseased bean seedlings. Sclerotia were obtained from a culture of the pathogen grown on potato dextrose agar (PDA) (7) at 27°C for 30 days. Sclerotia were collected after being air-dried at room temperature and tested for their viability by plating on PDA prior to each experiment. All the experiments were carried out using samples from one preparation of sclerotia.

Before each experiment, the pathogen was mixed with 100- to 250-g soil or sand samples that had been brought to the desired water content (1%, (wt/wt), in sand and 1.8% in Rehovot soil) (8) and preincubated for 24 h. Moistened soil or sand (5 g), containing the test material was poured into a nylon net bag. Bags were exposed to various sets of conditions in the apparatus described below, in triplicate.

Fumigation apparatus and procedure. A controlled-environment system was designed for this study, using a previously described fumigation apparatus (8). Narrow-neck glass containers (13 cm diameter, 25 cm high, 2 liter volume) were used as fumigation chambers. Each container was sealed with a glass lid and screw ring. The lid had two ports, both controlled by gas-tight valves (Elchem Plastic, Kibbutz Baram, Israel). Tubing (nylon 11; 5 mm diameter, 1-mm-thick wall) was used to connect the container to a pump for MB circulation and measurement. A rubber septum was installed in a third part located at the top of the

lid and used to inject the gas into the container. MB (100%, Dead Sea Bromine, Beer-Sheva, Israel) was injected as a gas into the container at a predetermined volume with a syringe. A calibration curve was used to determine the gas volume needed to achieve the appropriate MB concentration in the container atmosphere.

Methyl bromide was applied at rates ranging from 25 to 44 g m⁻³ air, as indicated. Concentrations of MB in the containers were measured with a gas analyzer (Fumiscopes-D, Statpower Technologies Corp., Burnaby, BC, Canada) equipped with a pump and thermal conductivity detector. The gas analyzer was connected to each container and the vessel atmosphere was circulated for 5 min through the detector, at a flow rate of 1 liter min⁻¹. Gas measurements were repeated at the end of the exposure period. At the end of each experiment, the containers were opened in a fumehood, and the MB was captured in an absorbent charcoal filter.

Assessing the viability of fungal populations. *S. rolfsii* sclerotia were retrieved from the bags by sieving, washed, and tested for viability by evaluating germination, as previously described (12). Stem peel pieces of sweet basil infected with *F. oxysporum* f. sp. *Basilici*, with typical pink spore masses, were recovered from the bags and agitated (Vortex-Genie, Springfield, MA) for 15 s at the highest setting in 2 ml of distilled water. The number of *F. oxysporum* f. sp. *basilici* macroconidia suspended in the water was adjusted to 5 × 10⁵ ml⁻¹ with a hemacytometer. Aliquots (100 µl) of the suspension were then plated on petri dishes with peptone-pentachloronitrobenzene *Fusarium* selective medium (7). Viability was assessed by incubating plates for 10 to 14 h at 27°C and determining the percentage of germination by direct microscopic examination.

Microbial counts by soil dilution. Each of three 5-g soil subsamples of each replicate (naturally infested with *F. oxysporum* f. sp. *basilici*, *F. oxysporum* f. sp. *radicis-lycopersici*, or *F. oxysporum* f. sp. *melonis*) was added to 45 ml of sterile water agar (0.1%, wt/vol) containing MgSO₄·7H₂O (0.1%, wt/vol). The samples were shaken for 15 min on a reciprocal shaker and then serially diluted with the same solution (down to 10⁻³ spores per ml). Samples of 0.2 ml were spread on five petri dishes that contained *Fusarium*-selective agar medium (7). Plates were incubated in the dark at 28°C for 4 to 6 days. Results are expressed as colony forming units per gram of soil (dried at 105°C for 48 h).

Heating apparatus and procedure. Propagules mixed with moistened sand were transferred to glass tubes (1.5 cm diameter, 2.5 ml). The tubes were sealed with Parafilm to minimize evaporation. Samples were heated in a water bath using a circulation heating system (Tep-3, Fried Electric, Haifa, Israel), with an accuracy of ± 0.1°C.

Exposure to soil microbial activity in laboratory applications. Propagules of *S. rolfsii* that were treated with MB or heat and untreated sclerotia were incorporated in pots filled with moistened Rehovot soil. The pots were kept in a controlled-temperature chamber at 27°C. A second set of treated samples, serving as a control, was kept in dry sterile sand.

Fumigation and solarization in field experiments. Four sets of field trials were carried out from 1995 to 1998 to test the effect of short solarization (8 days), fumigation with MB or metham sodium (Edigan, Agan-Makteshim Chemicals, Ashdod, Israel) at reduced doses, and a combination of the two treatments using two different application sequences. All experiments were carried out during the summer months (June through August) at the experimental farm of the Hebrew University in Rehovot, in sandy soil (3.8% clay, 95.7% sand, 0.5% organic matter, pH 7.9). Ambient and soil temperatures during the experimental period are given in Figure 1.

Plots (4 × 4 m) were outlined, and trenches (30 cm deep) were cut to bury the plastic edges. Gas-sampling tubing (nylon 11; 5 mm diameter, 5 m long) was placed in the center of each plot at

Fig. 1. Ambient and soil temperatures at three soil depths under plastic films or shading during fumigation with methyl bromide and solarization (July 1996).

various depths before the plastic tarps were laid, as previously described (12). The buried end of the tubing was protected with a porous plastic cylinder (10 cm long $\times$ 15 mm diameter). The other end was extended to an area outside the mulched plot. All plots were mulched with low-density polyethylene film (Polyon, Barkay, Israel). Fungal inocula were buried in the soil at the center of each plot before laying the plastic film. Mulched plots in which solarization was not desired during part or the duration of the experiments were shaded by covering with an Aluminet screen giving 90% shading capacity (Polysak, Barkay, Israel). The screen was stretched over the plots, leaving a 30 cm space over the plastic film. Treatments included short solarization (8 days), MB fumigation at low doses of 10 g m⁻² or metham sodium at 10 ml m⁻² (both under shading to prevent any effect of solarization), and a combination of each fumigant with solarization. Two sequences of combined applications were tested. Application of the first sequence was the fumigant under polyethylene film and shading screen for 48 h followed by removal of the screen and allowing an additional 8 days of solarization. The second sequence was initiated with 8 days of solarization followed by application of MB with shading of the plots for an additional 2 days. MB (Methabrom, 98% a.i., Dead Sea Bromine) was applied to preirrigated soil through drip lines that were placed under the film, using the hot gas method (19). MB concentration (*C*) in the soil was determined periodically following its application over time (*T*) during a 5-day period by connecting the sampling tubing to a 20-800 gas analyzer (GowMac Instrument Co., Bridgewater, NJ). Thus, *C* $\times$ *T* values were determined by calculating the area under the MB concentration times the time curve (12). Metham sodium was applied by drenching with 30 liters of water per m².

Statistical analyses. Each field experiment was conducted at least twice in the same area and with the same soil type. Separate analysis of each experiment showed homogeneous variances of

the experimental error between the repeats. Analysis of the data showed no significant interaction between the treatments and the replicate experiments; thus, only the effect of the treatments (e.g., gas concentration, mortality of fungal propagules) is shown for the analyses. The percentage of killing was arcsine-transformed before analysis. The effect of heat and MB were tested by two-way analysis of variance. The combinations of heating or solarization and MB or metham sodium were compared by one-way analysis of variance and interaction effects were tested by contrast *t* tests. Analysis for synergism was performed to evaluate fungal control by combined treatments, which was higher than the cumulative mortality level accomplished by each treatment alone. Synergism was expressed as the difference between mortality level accomplished by heating, solarization, or fumigant and the cumulative mortality value obtained by heating or solarization combined with fumigant. Inverse synergism indicated that the addition resulting from combination was less than additive. Significant difference from 0 was assessed using a *t* test. All treatment means were compared simultaneously by the Tukey test. All analyses were performed with the SAS program (release 6.04 for PC, SAS Software, Cary, NC) at *P* $\leq$ 0.05.

RESULTS

Effect of MB and heating at sublethal doses, alone or in combination, on pathogen viability. Exposure of *S. rolfssii* sclerotia to MB or heating affected rate and final percentage of germination (Fig. 2). Exposure of sclerotia to MB at 25 and 37.5 g m⁻³ or to heating at 48°C for 1 h resulted in a pronounced delay in germination, but the final percentage of germination was affected only at 37.5 g m⁻³. A similar trend of delayed germination by sublethal doses of MB and heating was obtained with macroconidia of *F. oxysporum* f. sp. *basilici* (results not shown). Based on these results and additional experiments, MB at 37.5 g m⁻³ and heating at 45°C for 2 h were used in the subsequent experiments as sublethal doses for treating *S. rolfssii* sclerotia. Exposure to heating or MB delayed germination, but after 48 h, germination percentages were similar to the untreated control (Fig. 3). However, combining heating and fumigation (in both application sequences) delayed germination and significantly reduced, by synergistic effect, the final percentage of germination. The sequence involving heating first followed by fumigation with MB was significantly more effective than the opposite sequence in delaying and reducing germination of *S. rolfssii* sclerotia. A similar

Fig. 2. The effects of methyl bromide (MB, 24 h of exposure) or heating (1 h of exposure) treatments under a controlled-environment system on germination of *Sclerotium rolfssii* sclerotia at three periods of incubation after treatment. For each MB concentration or heating treatment, columns topped with different letters are significantly different (*P* $\leq$ 0.05).

Fig. 3. The effects of methyl bromide (MB), heating (H), and combinations of both in a controlled-environment system on germination of *Sclerotium rolfssii* sclerotia. Con = nontreated sclerotia incubated at 25°C; H = heating at 45°C for 2 h; MB = exposure to MB at a concentration of 37.5 g m⁻³ of air for 24 h (concentration $\times$ time = 900 g h m⁻³); MB-H = application of MB followed by heating; H-MB = application of heating followed by MB. Values followed by different letters are significantly different (*P* $\leq$ 0.05). Bars indicate standard errors; + indicates significant synergistic effect in the related treatment (*P* $\leq$ 0.05).

trend was obtained with killing *F. oxysporum* f. sp. *basilici* macroconidia (Fig. 4). Thus, a synergistic mortality effect was obtained by combining heating and MB treatments with the appropriate sequence of application.

Weakening of sclerotia and exposure to soil microbial activity. The delayed germination of *S. rolfsii* sclerotia resulting from sublethal heating or fumigation and the increased mortality of propagules of the two tested pathogens by combined treatments suggested a weakening effect (11,18,20). Therefore, the effect of a further stress imposed on propagules that were previously treated with sublethal heating by exposure to soil microbial activity was examined.

Fig. 4. The effects of methyl bromide (MB), heating (H), and combinations of both in a controlled-environment system on survival of *Fusarium oxysporum* f. sp. *basilici* macroconidia. Con = incubation at 25°C; MB = exposure to MB at concentrations of 25 or 29.2 g m⁻³ of air for 24 h (concentration × time = 600 and 700.8 g h m⁻³, respectively); H = heating at 43°C for 1 h; MB-H = application of MB followed by heating; H-MB = application of heating followed by MB. For each MB concentration, columns topped with different uppercase or lowercase letters are significantly different ($P \leq 0.05$). + indicates significant synergistic effect in the related treatment ($P \leq 0.05$).

Fig. 5. Percent mortality of *Sclerotium rolfsii* sclerotia in a controlled environment after exposure to methyl bromide (MB), heating (H), and combinations of both in Rehovot soil. Con = incubation at 25°C; H = heating at 45°C for 2 h; MB = exposure to MB at a concentration of 37.5 g m⁻³ air for 24 h (concentration × time = 900 g h m⁻³); MB-H = application of MB followed by heating; H-MB = application of heating followed by MB. Within each set of propagule incorporation, columns topped with different uppercase letters are significantly different from each other ($P \leq 0.05$). Within each pair of columns, different lowercase letters are significantly different from each other ($P \leq 0.05$). + indicates significant synergistic effect in the related treatment ($P \leq 0.05$).

Previously MB- or heat-treated and untreated *S. rolfsii* sclerotia were incorporated into moistened Rehovot soil and incubated for 8 days. Sclerotial mortality was compared with that of similarly treated sclerotia that were kept in dry sterile sand for the same period. Results (Fig. 5) show that both heating and MB fumigation used alone, at the doses tested, were sublethal since they did not affect sclerotial viability (0 to 1% reduction in viability) when tested without exposure to soil microbial activity. However, soil incubation of heat or MB-treated sclerotia increased their mortality (up to 29%). As previously shown, the combined heating-MB treatment increased mortality of the propagules and was synergistic. Their incubation in the soil and exposure to soil microbial activity further increased their mortality to 93 to 100%. Again, the sequence of heating followed by MB treatment increased propagule sensitivity to the combined treatment. A similar trend was obtained at MB doses of 50 g m⁻³ (results not shown).

The effect of incorporating soil propagules that had been previously treated with low doses of MB was studied further in the field. Chlamydospores of *F. oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *radicis-lycopersici* were incorporated into moistened agricultural soil in the field at four depths and fumigated with MB. The mortality of half of the samples was assessed immediately after termination of fumigation (2 days after application); the mortality of the other half was assessed after an additional 8 days of incubation in the soil. MB treatment resulted in various levels of mortality, depending on soil depth and the pathogen (Fig. 6). However, extended incubation of propagules in the fumigated field soil further decreased the survival of the two pathogens in most tested depths, confirming the laboratory results (Fig. 5).

Combined effect of fumigants and short solarization in field tests. The higher effectiveness of combined treatments in the control of various pathogens was further examined under field applications. Combinations of short solarization and MB or

Fig. 6. Percent mortality of pathogen propagules in the field after low-dose (10 g m⁻³) fumigation with methyl bromide (MB) (concentration × time values for 10, 20, 30, and 40 cm depths were 245, 221, 181, and 153, respectively). MB-2 = Sample assessed immediately after termination of fumigation (2 days after application); MB-10 = assessment was done 10 days after application; *F. o. melonis* = chlamydospores of *Fusarium oxysporum* f. sp. *melonis* produced in soil; FORL = soil naturally infested with *F. oxysporum* f. sp. *radicis-lycopersici*. For each pathogen, at every depth, columns topped with different letters are significantly different ($P \leq 0.05$). Mortality was calculated by comparing with nontreated propagules.

metham sodium, another common soil fumigant, both at low doses, were examined. MB at 10 g m^{-2} or metham sodium at 10 ml m^{-2} are considered sublethal doses, since they are equivalent to $\approx 20\%$ of the recommended dose for field application. Indeed, monitored $C \times T$ values were sublethal, as also shown previously (8). In most cases, the combined treatments were more effective than each treatment alone in controlling the four tested pathogens (Fig. 7). In certain cases, the combined treatment was synergistic, but there were also some instances of inverse synergism, namely, the effect was less than additive. With certain combinations, the sequence of solarization first followed by MB treatment was significantly more effective than the opposite treatment sequence. These findings again confirmed the results obtained under controlled conditions (Figs. 3 through 5). A similar trend with respect to the effect of application sequence was obtained with metham sodium (Fig. 8). At a depth of 10 cm, both fumigants were highly effective even when each was applied alone, appar-

ently due to the higher effectiveness of the treatments at that depth.

DISCUSSION

The optimal use of a combination of control agents is at the heart of a successful integrated pest management program, since it has the potential to improve pest control and broaden the spectrum of affected pests, reducing the dose of pesticide needed and extending the effectiveness of the treatments. Indeed, in our experiments, effective control of soilborne fungi was obtained using low doses of the control agents, with short exposure to heating or solarization, which were sublethal when used alone. The combination of solarization with other methods of control should be considered from two points of view: as a way of improving solarization, e.g., by shortening the length of the application from the standard 30 to 40 days to 8 days, and, as a

Fig. 7. The effect of combined exposure to methyl bromide (MB) at a low dose (10 g m^{-2}) and short solarization (S, 8 days) on percentage of propagule mortality at four depths in the field. Concentration $\times$ time values for 10, 20, 30, and 40 cm depths were 261, 237, 208, and 167, respectively. Con = untreated control; MB-S = fumigation followed by solarization; S-MB = solarization followed by fumigation; FOBA = macroconidia from stems of diseased sweet basil naturally infected with *Fusarium oxysporum* f. sp. *basilici*; *S. rolfsii* = sclerotia of *Sclerotium rolfsii*; *F. o. melonis* = chlamydospores of *F. oxysporum* f. sp. *melonis* produced in soil; FORL = soil naturally infested with *F. oxysporum* f. sp. *radialis-lycopersici*. For each pathogen, columns topped with different letters are significantly different ($P \leq 0.05$); + indicates significant synergistic effect in the related treatment; - indicates significant inverse synergism ($P \leq 0.05$).

way of improving the other methods with which solarization is combined (17). From the latter point of view, a suitable combination of solarization with a reduced dose of a chemical pesticide should reduce the pesticide's harmful effects (and, thus, make it more acceptable) without reducing control effectiveness, but rather increasing it.

The present study shows that combining heating or short solarization with a reduced dose of either MB or metham sodium is more effective than each treatment alone in controlling a variety of fungal pathogens. A synergistic effect was frequently observed (Figs. 3 through 5, 7, 8). This was verified both under controlled conditions and in the field. For example, application of heating or MB under controlled conditions at doses that reduced the fungal pathogen by only 0 to 10% resulted in up to 90% pathogen control when appropriately combined (Figs. 3 through 5). Similarly, neither MB at 20% of the recommended dose nor a short solarization of 8 days could control the populations of *F. oxysporum* f. sp. *basilici* at a depth 20 cm or *S. rolsii* at a depth of 40 cm under field conditions, while combining them in the appropriate sequence resulted in 100% reduction (Fig. 7). A

similar trend was obtained with metham sodium at 20% of the recommended dose (Fig. 8). Incubation of the propagules in soil (i.e., exposure to the activity of resident soil microorganisms) further improved pathogen control (Fig. 5)—a principle that was also verified under field conditions (Fig. 6). These findings also have practical applications: a period of time should be allowed between the termination of fumigation and planting to enable a further reduction in pathogen population. Thus, an assessment of pathogen populations immediately after treatment may not reflect the full potential of the treatment in terms of pathogen control. The lower effectiveness of fumigation, which has been encountered in certain cases, may be due to the fact that planting was carried out shortly after fumigation.

When pathogen populations are exposed to sublethal doses of a control agent, the surviving propagules are stressed and weakened and may become more vulnerable to other biotic or abiotic agents. This weakening effect is the basis of the synergistic action of combined treatments (18). We showed that sublethal doses of MB or heating, applied in a controlled environment system, delayed germination of *S. rolsii* sclerotia (Fig. 2), which is a typical sign of weakening (20). The same trend has been obtained with *F. oxysporum* f. sp. *niveum* conidia, whose germ-tube elongation was reduced as a result of weakening by heat (11). Slower germination in the soil would extend the time during which the vulnerable germ tubes are exposed to hostile microbial activity. This diminishes the chance of the propagules successfully penetrating the plant tissues. Lifshitz et al. (20) found that sublethal heating increases the leakage of organic substances from sclerotia and subsequently stimulates invasion of the sclerotia by soil microorganisms, eventually leading to their decay. Similarly, heated chlamydospores of *F. oxysporum* f. sp. *ciceri* lost organic compounds, were more readily colonized by soil microorganisms, and sustained a further loss of viability and pathogenic aggressiveness (2). Germination and melanization of sublethally heated microsclerotia of *Verticillium dahliae* were slowed (23). The increased mortality rate of pathogen propagules treated with sublethal doses of fumigants or heating and subsequently incubated in soil (Figs. 5,6) may be attributed to similar mechanisms. Pretreatment of pathogen propagules by a sublethal dose of MB increased pathogen mortality, caused by soil microorganisms (Fig. 5). Thus, weakening of the propagules by sublethal treatment appears to trigger enhanced biological control by soil organisms by increasing their vulnerability. Enhanced colonization of weakened propagules by a *Trichoderma* was demonstrated long ago with *S. rolsii* treated with formalin (6) and with an *Armillaria* treated with CS₂, another mild fumigant (4). Garrett (15) thoroughly discussed this phenomenon, but unfortunately, the potential of this approach was not exploited, apparently due to the availability of powerful fumigants.

The sequence of application had a significant effect on the improved control of pathogens, as demonstrated in all tested systems (Figs. 3 through 5, 7, 8). Sublethal heating of sclerotia of *S. rolsii* caused cracks in the sclerotia (20), which may facilitate the penetration of MB to the sclerotia and may explain why a sequence of first heating and then fumigating is more effective than the opposite sequence. The sequence of application should be thoroughly considered when planning treatment combinations. One practical recommendation based on our findings is to solarize the soil for a short period and later, to introduce the desired fumigant or biocontrol agent via a drip-irrigation system or other means.

Combining solarization with other control agents may enable a shortening of the solarization period. Thus, the solarization-metham sodium combination killed more propagules of *F. oxysporum* f. sp. *vasinfectum* faster than solarization alone (3). Additional benefits of combining solarization with pesticides might include extending the retention of volatile pesticide trapped under the plastic mulch (14) and, possibly, activation of the

Fig. 8. The effect of combined exposure to metham sodium (MS) at low-dose (10 ml m⁻²) fumigation and short solarization (S, 8 days) on percentage of propagule mortality at three depths in the field. Con = untreated control; MS-S = fumigation followed by solarization; S-MS = solarization followed by fumigation; FOBA = stems of diseased sweet basil naturally infected by *Fusarium oxysporum* f. sp. *basilici*; FORL = soil naturally infested with *F. oxysporum* f. sp. *radicis-lycopersici*. For each pathogen, columns topped with different letters are significantly different ($P \leq 0.05$). + indicates significant synergistic effect in the related treatment; - indicates significant inverse synergism ($P \leq 0.05$).

pesticide when heated (1). The beneficial effect of combining solarization, heating, or fumigants with other control agents under field conditions has been demonstrated with a variety of crops and pathogens (3,5,9,13,14,21–23). For example, combining metham sodium at a sublethal dose with a biocontrol agent reduced infection of eggplant by *V. dahliae* (10).

The present study shows that MB and metham sodium doses can be reduced, solarization can be shortened, and the effectiveness of pathogen control increased by properly combining the control agents. This approach should also be adopted with other pesticides and control agents and can be followed to develop technologies that are environmentally acceptable and more effective for pest management. This is especially necessary at present, when attempts are being made to replace MB with nonchemical methods rather than other fumigants, and consumption of this fumigant has to be immediately reduced.

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LITERATURE CITED

- Aharonson, N., and Katan, J. 1991. Pesticide behavior in solarized and disinfected soils. Pages 131-138 in: *Soil Solarization*. J. Katan and J. E. DeVay, eds. CRC Press, Boca Raton, FL.
- Arora, D. K., Pandey, A. K., and Srivastava, A. K. 1996. Effects of heat stress on loss of C, germination and pathogenicity for chlamydospores of *Fusarium oxysporum* f. sp. *ciceri*. *Soil Biol. Biochem.* 28:399-407.
- Ben-Yephet, Y., Melero, J. M., and DeVay, D. E. 1988. Interaction of soil solarization and metham-sodium in the destruction of *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *vasinfectum*. *Crop Prot.* 7:327-331.
- Bliss, D. E. 1951. The destruction of *Armillaria mellea* in citrus soils. *Phytopathology* 41:665-683.
- Chellemi, D. O., Olson, S. M., and Mitchell, D. J. 1994. Effects of soil solarization and fumigation on survival of soilborne pathogens of tomato in northern Florida. *Plant Dis.* 78:1167-1172.
- Davey, A. E., and Leach, L. D. 1941. Experiments with fungicides for use against *Sclerotium rolfsii* in soils. *Hilgardia* 10:523-547.
- Dhingra, O. D., and Sinclair, J. B. 1986. *Basic Plant Pathology Methods*. CRC Press, Boca Raton, FL.
- Eshel, D., Gamliel, A., Katan, J., and Grinstein A. 1999. Evaluation of soil fumigants on soilborne fungal pathogens in a controlled-environment system and in soil. *Crop Prot.* 18:437-443.
- Frank, Z. R., Ben-Yephet, Y., and Katan, J. 1986. Synergistic effect of metham and solarization in controlling delimited shell spots of peanut pods. *Crop Prot.* 5:199-202.
- Fravel, D. R. 1996. Interaction of biocontrol fungi with sublethal rates of metham sodium for control of *Verticillium dahliae*. *Crop Prot.* 15:115-119.
- Freeman, S., and Katan, J. 1988. Weakening effect on propagules of *Fusarium* by sublethal heating. *Phytopathology* 78:1656-1661.
- Gamliel, A., Grinstein, A., Klein, L., Cohen, Y., and Katan, J. 1998. Permeability of plastic to methyl bromide: Field study. *Crop Prot.* 17:241-248.
- Gamliel, A., Hadar, E., and Katan, J. 1993. Improvement of growth and yield of *Gypsophila paniculata* by solarization or fumigation of soil or container medium in continuous cropping systems. *Plant Dis.* 77:933-938.
- Gamliel, A., and Stapleton, J. J. 1993. Effect of chicken compost or ammonium phosphate and solarization on pathogen control, rhizosphere microorganisms, and lettuce growth. *Plant Dis.* 77:886-891.
- Garrett, S. D. 1956. *Biology of Root-Infecting Fungi*. Cambridge University Press, Cambridge.
- Grinstein, A., Kritzman, G., Hetzroni, A., Gamliel, A., Mor, M., and Katan, J. 1995. The border effect of soil solarization. *Crop Prot.* 14:315-320.
- Katan, J. 1996. Soil solarization: Integrated control aspects. Pages 250-278 in: *Principles and Practice of Managing Soilborne Plant Pathogens*. R. Hall, ed. American Phytopathological Society Press, St. Paul, MN.
- Katan, J., Ginzburg, C., and Freeman, S. 1992. The weakening effect as a trigger for biological control and criteria for its evaluation. Pages 55-61 in: *Biological Control of Plant Diseases*. E. C. Tjamos, G. Papavizas, and R. Cook, eds. Plenum Press, New York.
- Klein, L. 1996. Methyl bromide as a soil fumigant. Pages 191-235 in: *The Methyl Bromide Issue*. C. H. Bell, N. Price, and B. Chakrabarti, eds. Wiley and Sons, New York.
- Lifshitz, R., Tabachnik, M., Katan, J., and Chet, I. 1983. The effect of sublethal heating on sclerotia of *Sclerotium rolfsii*. *Can. J. Microbiol.* 29:1607-1610.
- Ristaino, J. B., Perry, K. B., and Lumsden, R. D. 1991. Effect of solarization and *Gliocladium virens* on sclerotia of *Sclerotium rolfsii*, soil microbiota, and the incidence of southern blight of tomato. *Phytopathology* 81:1117-1124.
- Sivan, A., and Chet, I. 1993. Integrated control of *Fusarium* crown and root rot of tomato with *Trichoderma harzianum* in combination with methyl bromide or soil solarization. *Crop Prot.* 12:380-386.
- Tjamos, E. C., and Fravel, D. R. 1995. Detrimental effects of sublethal heating and *Talaromyces flavus* on microsclerotia of *Verticillium dahliae*. *Phytopathology* 85:388-392.