Fungicidal Control of *Sclerotinia sclerotiorum* in Soil with a Combination of Benomyl and Thiram

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ABSTRACT

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In previous experiments, the fungicide thiram suppressed benomyl degradation and prolonged its activity in the soil. Combined application of benomyl and thiram improved control of *Sclerotinia sclerotiorum* in soil. The effect of the combined fungicidal treatments was expressed by a reduction of sclerotial germination, apothecial production by germinating sclerotia, rate of apothecial production, and delay in apothecial emergence. A reduction in apothecial emergence as a result of the combined treatment was also observed in the field. Improved control of Verticillium wilt was also observed under field conditions. The low mobility of benomyl in the soil profile combined with the extended persistence of fungitoxic residues resulting from the thiram amendment contribute to the improved control of the pathogen by benomyl in soil while providing an option for benomyl dosage reduction. Thiram may prove useful as an extender and fortifier of benomyl activity in soil.

Additional key words: benzimidazole fungicides, carbendazim, chinese cabbage, iprodione, lettuce, vinclozolin

Several pesticides are used worldwide to control *Sclerotinia* spp. One of the most common is the fungicide benomyl (9), which when applied to foliage and soil, controls sclerotia, apothecia, and ascospores. The success of benomyl treatments applied to soil can be diminished by loss of fungicidal activity because of microbial degradation of the compound in soil (11). In earlier laboratory studies, we have shown that the fungicide thiram (also known as TMTD), when applied to soil along with benomyl, delays degradation of the latter, thus prolonging persistence of the compound's fungicidal activity in soil (11). Other fungicides, e.g., iprodione and vinclozolin (5), are also effective in controlling *Sclerotinia* spp.

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The purpose of this study was to investigate whether a combination of thiram and benomyl applied to soil can extend fungicide persistence and increase effectiveness to simultaneously improve control of *Sclerotinia sclerotiorum* (Lib.) de Bary under outdoor and field conditions and reduce benomyl application dosage.

MATERIALS AND METHODS

Fungicides, soils, and chemicals. Commercial formulations of the following fungicides were used: benomyl (Benlate 50WP), thiram (Tirpa 80WP), thiabendazole (Pitrizet 30WSC), vinclozolin (Ronilan 50WP), and iprodione (Rovral 50WP).

Characteristics of soils in field trials were: Gilat, silty loessial loam (0.5% organic matter, 20% clay, pH 8.4, field capacity 20%, v/w); Sharsheret, loess (0.5% organic matter, 23% clay, pH 8.8, field capacity 21%, v/w); Gilgal, loamy clay (0.5% organic matter, 25% clay, pH 7.8, field capacity 32%, v/w). Gilat silty loessial loam soil was used for outdoor experiments. Rehovot sandy loam soil (0.6% organic matter, 3.8% clay, pH 7.9, field capacity 9%, v/w) was used for determining benomyl mobility in the soil profile.

Chemicals used were: ethyl acetate and methanol (analytical), glass-redistilled, from Frutarom (Israel), as were HCl, NaOH, and NH_4OH (analytical). Anhydrous Na_2SO_4 (analytical) and dimethyl sulfoxide (DMSO) were from Merck (West Germany).

Outdoor experiments. All outdoor experiments were performed in a shade-covered area at the Faculty of Agriculture, Rehovot. Sclerotia of *S. sclerotiorum* recovered from infected eggplant (*Solanum melongena* L.) were placed 1 cm below the soil surface in pots (9 × 11 × 6 cm), 10 sclerotia per pot. Each day, the pots were watered carefully to avoid flooding and uncovering sclerotia. Fungicides were applied 1 wk after placing sclerotia. Benomyl was applied at rates equivalent to 6.25, 12.5, 25, 50, and 100 $\mu g/cm^2$ of surface area. Thiram was applied at 25 and 50 $\mu g/cm^2$. Application was in aliquots of 10 ml per pot, using a syringe with a hypodermic needle bent to 90° at its edge to simulate a fan-jet sprayer nozzle. Five replicates of each treatment were arranged in a randomized block design. Recordings of sclerotial germination, i.e., emergence of apothecia at the soil surface, and number of apothecia per germinating sclerotium were carried out daily. Eighty days after fungicide application, the soil in each pot was mixed and samples were taken and stored at −18 C until fungicide residue was analyzed.

Fungitoxic tests of benomyl and thiram on *S. sclerotiorum* in culture. Fungicide solutions were prepared in DMSO. Aliquots of solutions (60 μl) were added to 20 ml of potato-dextrose agar (PDA) (about 50 C), mixed, and poured into petri dishes. Final concentrations of the fungicide were 0.1–5.0 μg a.i./ml of medium. Fungicides were added to the medium either separately or in various combinations. Sclerotia of *S. sclerotiorum* (5–10 mm long) were surface-disinfected (2 min in 1% sodium hypochlorite followed by two rinses in

sterile tap water) and placed, three sclerotia per dish, in four replicates of each fungicide-amended dish. The number of germinating sclerotia was recorded after 4 days of incubation (14 C).

Extraction and chemical analysis of fungicide residues in soil. To determine the possible relationship between improved control of *S. sclerotiorum* in outdoor experiments and the persistence of carbendazim (a fungitoxic hydrolysis product of benomyl), this substance was extracted from the experimental pots and quantitatively analyzed. The extraction procedure was as follows: A soil sample (20 g) was shaken in an Erlenmeyer flask with 1 N NaOH (200 ml) for 10 min. The aqueous solution was separated from the soil and extracted three times, with 150, 100 and 100 ml, respectively, of ethyl acetate. The ethyl acetate extracts were combined, concentrated in a rotary evaporator to about 75 ml, and extracted with 75, 50, and 50 ml of 0.1 N HCl. Fifty milliliters of 3 N NaOH was added to the aqueous phase, which was then extracted with 100, 80, and 80 ml of ethyl acetate. The ethyl acetate extract was dried with anhydrous sodium sulfate, evaporated to dryness, and the residues were dissolved in methanol. Aliquots were injected into an apparatus composed of a Tracor 985 HPLC and Tracor 951 LC pump connected to a Perkin Elmer 204 fluorescence spectrophotometer set at 285 nm excitation and 307 nm for carbendazim analysis. The HPLC was equipped with a C-18 reversed phase (10 μm) packed in a 20-cm-long (4 mm i.d.) column. The solvent used was methanol + H_2O (4:1, v/v) and ammonium hydroxide at 10 drops per liter. Flow rate was 0.5 ml/min.

Movement of benomyl in soil. To determine movement of benomyl in soil, Rehovot sandy loam soil (2.5 kg) at 75% field capacity was placed in a rectangular container (10 × 10 × 25 cm) open at one end and blocked by a sieve at the other (to contain soil but allow excess water to flow out). Removable seams along two of the container's sides facilitated halving the container along with its contents when required. Aliquots (10 ml) of benomyl suspension (200 mg/L) were sprayed on the soil surface, then the same surface was sprayed with 250 ml of tap water (equivalent to 25 mm of rainfall). Seventy-two hours after fungicide application, the containers (three replicates) were halved and 200-mg soil samples were removed from microsites along the center of the profile now exposed (thus minimizing the effect of variation in distribution expected along the container walls). Samples were taken from various depths at intervals ranging between 0.5 and 2.0 cm. Concentration of fungitoxic residues (by the time samples were analyzed, most of the benomyl had been hydrolyzed to carbendazim) was determined by soil-agar pellet bioassay, which permits assessment of fungitoxic residues while using only minute soil samples.

Determination of carbendazim in soil by soil-agar pellet bioassay. The soil-agar pellet bioassay (10) was used to determine benomyl movement into the soil profile. Pellets composed of mixtures of soil (200 mg) and 0.3 ml of agar (30 g/L) were placed on PDA (with 250 μg/L chloramphenicol) preinoculated with conidia of the test organism (*Penicillium digitatum* Sacc.). After cold preincubation followed by incubation at 27 C, the size of the fungus inhibition zone was determined and compared with a standard curve of inhibition by known carbendazim concentrations.

Field trials. Experiments in the field were carried out during the winter months of 1984 and 1985 at three locations (Sharsheret, Gilat experimental station, and Gilgal experimental station). Lettuce (*Lactuca sativa* L. 'Crisp Head') was planted on 6 November in a field at Sharsheret that was naturally infested with sclerotia of *S. sclerotiorum* and in artificially infested plots (about 170 sclerotia per square meter scattered by hand and incorporated into the soil with a rotary cultivator to a maximum depth of 15 cm) in Gilat. On 26 November, lettuce was planted in another artificially infested field at Gilgal. One month after planting, benomyl suspensions were applied at rates of 2.5 and 5.0 kg/ha. Thiram was applied at the rate of 5.0 kg/ha. Suspensions were prepared in garden watering cans (14 L of water) and applied to five replicate plots (5.0 × 1.2 m) arranged in a randomized block design. At Sharsheret, the field trial also included application of three other fungicides (thiabendazole, iprodione, and vinclozolin) at the rate of 5.0 kg/ha. At various time intervals throughout the growing season, the number of sclerotia germinating to give apothecia within the 2-m center area of the test plots was recorded.

In another field trial at Sharsheret, chinese cabbage (*Brassica pekinensis* (Lour.) Rupr. '70') was sown on 25 September 1984 in a field naturally infested with *Verticillium dahliae* Kleb. Two weeks after sowing, benomyl and thiram were applied at the rates of 10.0 and 5.0 kg/ha, respectively. The fungicides were applied as drench treatments as previously described either separately or as combined treatments to five replicates of the randomized block experiment. Twelve weeks after sowing, 25 cabbage heads in each plot were cut at soil level. Browning of vascular plant tissue at the cut was presumed to indicate *V. dahliae* incidence. This was further verified by placing surface-disinfected (3 min in 1% sodium hypochlorite followed by two rinses in sterile tap water) cuttings on selective medium (3) at 20 C for about 2 wk, until fungus growing from the cuttings was identifiable. At the end of the experiment, cabbage head quality was tested for increase in exportable produce.

RESULTS

Effects of benomyl and thiram soil drench treatments on *S. sclerotiorum* in soil in outdoor experiments. Thiram alone slightly reduced apothecial production. Benomyl reduced apothecial production, showing a dose response at higher concentrations (Fig. 1). Combining benomyl with thiram resulted in a more pronounced reduction in apothecial production. This trend of increased fungicide effectiveness with the combined treatments was observed in an additional experiment similarly designed. At 50 and 100 μg/cm^2 benomyl, apothecial production was completely inhibited.

Apart from number of apothecia produced, other parameters were used to study the effects of the fungicides. The slope of the linear phase of apothecial appearance over time was used to determine the rate of apothecial production. Comparison of the slopes (as derived from linear regression analysis of about 12 days of daily apothecial appearance recordings) reveals that neither benomyl nor thiram alone affected the rate of apothecial production. However, all six combinations of benomyl with thiram resulted in a lower rate of apothecial production compared with benomyl alone (Table 1). Reduction in the total number of apothecia, caused by the fungicides (Fig. 1), could result from either a decrease in the number of sclerotia that produce apothecia (germination) or from an effect on number of apothecia produced by each germinating sclerotium or from a combination of both. Thus, effects of the treatments on sclerotial germination and on the mean number of apothecia produced by the germinating sclerotia was also determined. Results (Table 1) show that when applied alone, benomyl applied at the higher rates reduced sclerotial germination. Thiram alone only slightly affected this parameter. Combinations of the two fungicides reduced sclerotial germination. Benomyl, when applied alone, did not affect the number of apothecia produced even at a concentration of 25 μg/cm^2, which reduced their total number by 81% (Fig. 1). In contrast, the benomyl-thiram combinations were effective in reducing the number of apothecia produced by each germinating sclerotium by 13% at the lowest concentration (effect not significant) to 61% at the highest concentration applied. Both inhibition of sclerotial germination and a decrease of apothecial production by the germinating sclerotia was evident at the benomyl application rate of 12.5 μg/cm^2 combined with thiram at the two rates applied.

The fungicides also delayed the emergence of apothecia from sclerotia. Data were recorded as the day of appearance of apothecia derived from the second sclerotium germinating to give

fruiting bodies in each treated pot. High variability of sclerotial germination led us to choose the second sclerotium rather than the first in order to lessen the probability of misjudging the results because of occasional germination of the first sclerotium. With increased benomyl application rates, apothecial appearance was increasingly delayed (Table 1). This delay was more pronounced when fungicides were combined, especially at the lower benomyl rates. The higher doses of benomyl used were independently effective, possibly masking the supporting effect of thiram in controlling apothecial appearance (Table 1). These results led us to further investigate the effects of the separate and combined benomyl and thiram treatments on the pathogen in culture.

Effect of benomyl and thiram on *S. sclerotiorum* in culture. Preliminary tests showed no effect of the solution solvent (DMSO) on sclerotial germination at twice the solvent concentration used throughout the experiment. Benomyl concentration of 0.78 μg/ml inhibited germination of 50% of the tested sclerotia, whereas the ED_{50} of thiram was much higher (3.1 μg/ml). In all effective fungicide combinations tested, the combined treatments resulted in additive effects with no indication of any interaction between the chemicals, thus eliminating the likelihood of synergistic effects in the fungicidal activity of the two combined compounds.

Effect of thiram on persistence of benomyl in soil. Eighty days after fungicide application in outdoor experiments, soil samples from the benomyl (25 μg/cm²) and the combined benomyl and thiram (25 and 50 μg/cm², respectively) treatments were removed, extracted, and quantitatively analyzed for carbendazim residues. In the benomyl-treated soil, carbendazim residues did not exceed 0.08 μg/g of soil, whereas in the thiram-amended, benomyl-treated soil, carbendazim persisted at a fungitoxic concentration of 0.31 μg/g.

Movement of benomyl applied as drench treatment in soil. Benomyl was applied as a surface spray of soil in containers and was incorporated by irrigation. Thus, the compound's movement in the soil through irrigation was followed in a simulation system. Results determined by the soil-agar pellet bioassay (Fig. 2) show that benomyl only slightly penetrated the soil profile. Most of the detected fungitoxic compound (74%) remained within the upper 2.5 cm of the soil profile. There was some movement to deeper layers; 19% was found between 2.5 and 4.5 cm, but only a minor portion (6.5%) was found between 4.5 and 6.5 cm deep.

Effects of soil-applied fungicides on apothecial production under field conditions. The effects of benomyl, thiram, and their combinations applied as soil drench treatments were followed in three field trials with lettuce (Table 2). Benomyl or thiram reduced the total number of apothecia in two of the three locations where experiments were conducted. The combined benomyl-thiram treatments further reduced the total number of germinating sclerotia. Although not statisticaly significant, there was an obvious trend of improved control with the combination of the two fungicides in all instances at the three experimental sites. Soil drench applications of other fungicides (thiabendazole, iprodione, and vinclozolin), not commonly used in Israel for control of *S. sclerotiorum*, were also tested at the field trial in Sharsheret. These three fungicides were as effective as benomyl at the same application rate in reducing the number of sclerotia producing apothecia in the field. Thus, the effects of these fungicides were similar to that of benomyl applied at 5.0 kg/ha, reducing sclerotial germination to yield apothecia to 35–40% of that observed in untreated plots.

Effect of benomyl and thiram on incidence of *V. dahliae* in chinese cabbage. The effectiveness of benomyl-thiram combinations in controlling another sensitive pathogen was examined under field conditions. When applied as a combined soil drench 2 wk after seeding, the two fungicides reduced *V. dahliae* incidence by 45% in chinese cabbage grown in a field naturally infested with the pathogen. Again, treatment with the fungicidal combinations was more effective than treatment with the fungicides alone and had the largest difference from the control. In more than 85% of the observations in the field, where disease incidence was determined visually (browning of vascular tissue at

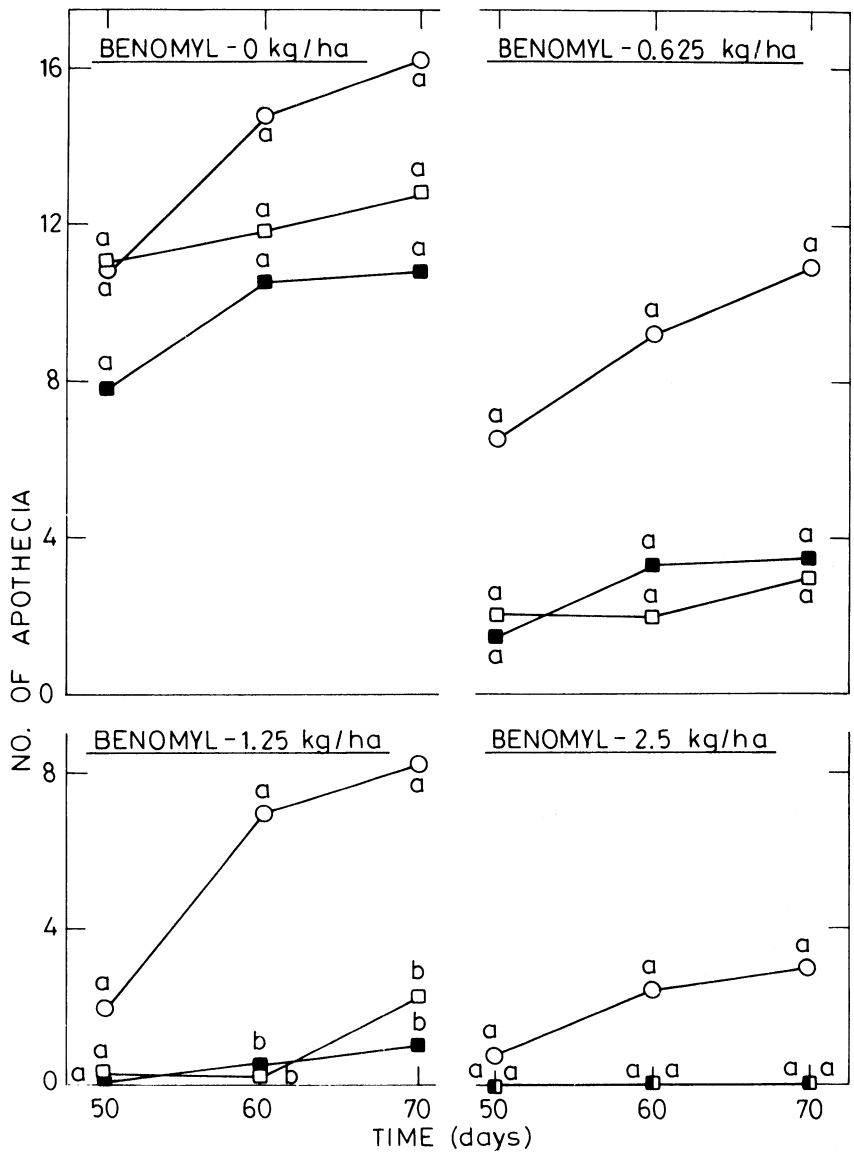

Fig. 1. Effects of benomyl (o——o) and combined application of benomyl with the equivalents of 2.5 kg/ha (□——□) and 5.0 kg/ha (■——■) thiram to soil on apothecial appearance (cumulative) in an outdoor experiment. Points indicate mean of five replicates and are part of daily observations throughout the experiment.

Table 1. Effects of benomyl, thiram, and combined drench application of the two fungicides on *Sclerotinia sclerotiorum* in soil as expressed in different parameters

Treatment (kg/ha)	Rate of apothecial production[a,b] (slope)	Sclerotial germination[b,c] (%)	Mean number of apothecia per germinating sclerotium[c,d]	Mean day of germination of second sclerotium[b,e]
Untreated	0.91	62.5	2.50	5.75
Thiram (2.5)	0.87	40.0	2.90	4.75
Thiram (5.0)	0.83	40.0	2.77	7.00
Benomyl (0.625)	0.98	42.5	2.47	8.25
Benomyl (0.625) + thiram (2.5)	0.40	12.5	2.17	22.50
Benomyl (0.625) + thiram (5.0)	0.31	17.5	1.67	25.50
Benomyl (1.25)	0.83	32.5	2.55	12.75
Benomyl (1.25) + thiram (2.5)	0.37	20.0	1.00	20.75
Benomyl (1.25) + thiram (5.0)	0.49	7.5	1.33	30.00
Benomyl (2.5)	0.44	15.0	2.10	28.00
Benomyl (2.5) + thiram (2.5)	NG[f]	NG	NG	NG
Benomyl (2.5) + thiram (5.0)	NG	NG	NG	NG

[a] Slope was determined by linear regression analysis of cumulative apothecial number in time throughout the linear phase of apothecial appearance. All correlation coefficients (r) ranged 0.90–0.98 and were significant at a 95% confidence level.
[b] Factorial analysis showed that all main effects of benomyl and thiram were significantly different from untreated soil ($P = 0.05$). Interactions between treatments were not significant.
[c] Only apothecia producing sclerotia were considered germinated.
[d] Factorial analysis showed that, excluding benomyl at 0.625 kg/ha, all main effects of both fungicides differed significantly from untreated soil ($P = 0.05$). Interactions between treatments were not significant.
[e] Days from second sclerotium germination to give apothecia in untreated soil. No germination was given the number 33, because this was the day after the last recording of apothecial germination.
[f] NG = No germination of sclerotia to produce apothecia.

Table 2. Effects of benomyl, thiram, and combined drench application of the two fungicides on number of sclerotia of *Sclerotinia sclerotiorum* germinating to produce apothecia in lettuce fields at three locations and on *Verticillium dahliae* incidence in a chinese cabbage field

Treatment (kg/ha)	Apothecia incidence at three lettuce fields (% of untreated)[a]			*Verticillium* incidence in chinese cabbage (% of untreated)[b]
	Sharsheret	Gilat	Gilgal	Sharsheret
Untreated	100	100	100	100
Thiram (5.0)	76	88	121	76
Benomyl (2.5)	83	45	95	NA[c]
Benomyl (5.0)	42	51	106	NA
Benomyl (10.0)	NA	NA	NA	70
Benomyl (2.5) + thiram (5.0)	45	33	73	NA
Benomyl (5.0) + thiram (5.0)	38	29	65	NA
Benomyl (10.0) + thiram (5.0)	NA	NA	NA	55

[a] Actual apothecial number in the field averaged 19, 20, and 16 apothecia per square meter of untreated plots at the respective fields. Factorial analysis showed that, excluding Gilgal, main effects of benomyl and thiram were significantly different from untreated plots ($P = 0.05$). Interactions between treatments were not significant.
[b] Incidence in the untreated plots averaged 34% infected plants. Factorial analysis showed no significant difference between fungicide-treated and untreated plots.
[c] NA = Not applied.

the cabbage head cut), further laboratory tests verified the presence of *V. dahliae*. An increase of about 10% in export-quality cabbage heads was observed in all plots where fungicides were applied.

DISCUSSION

The potential advantages of combining pesticides (or of any integrated control approach) would be improved control, extended effectiveness, and reduced dosages of the major pesticide. Several parameters were chosen to demonstrate the possibility of improved control by combining benomyl and thiram. A delay in apothecial appearance was achieved, resulting in a shortened period of host exposure to the pathogen throughout the growing season. The reduction in apothecial production by the combined treatment is the result of a reduction in both the number of apothecia produced per germinating sclerotium and the percentage of sclerotia germinating to yield apothecia. Our results do not indicate a synergistic fungicidal effect by the fungicide combinations. However, the combined treatment was better, in most cases, than either fungicide alone. Thiram has been shown to extend benomyl persistence in soil, as found in an earlier study (11), as well as that of the herbicide terbutryn (4). Thus, thiram may be considered an extender, similar in function to the compound R-33865, which extends the activity of the herbicide EPTC (6). Furthermore, thiram has some independent fungicidal effect on *S. sclerotiorum* and thus may also be described as a fortifier of benomyl activity. Such fortifiers have obvious prospects for dealing with short-life, nonpersistent pesticides like benomyl.

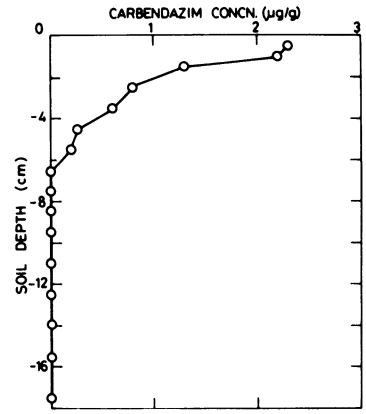

Fig. 2. Distribution of benomyl and fungitoxic residues in soil profile. Benomyl was sprayed on the soil surface, then water was sprayed at the equivalent of 25 mm of rainfall. Fungicide was analyzed by soil-agar pellet bioassay.

In addition to the three advantages resulting from combined fungicidal control that were verified in this study, there may be others. A combined treatment provides a wider spectrum of control. Indeed, thiram has advantages in this respect because it also controls other soilborne pathogens and may even render the soil suppressive to *Pythium* (7). Reducing benomyl dosage may reduce the chances for negative side-effects, e.g., suppression of nontarget beneficial microorganisms, development of resistant biotypes, and the buildup of microbial populations, which are responsible for enhanced degradation and loss of effectiveness of benomyl. In this study, amending benomyl with thiram allowed a 50% reduction in benomyl application rates without loss in effectiveness of *S. sclerotiorum* control.

Changing methods of application may also improve control. Benomyl soil treatments usually require larger doses than foliar applications and are somewhat less effective systemically. However, application is simpler (no need to reenter the field several times during the growing season) and chemicals affect inoculum in critical niches that aboveground applications are less likely to reach, such as

beneath plant foliage. The fact that benomyl and carbendazim have relatively low mobility in soil (1,8) can be advantageous when attempting to control *S. sclerotiorum* in soil. Sclerotial germination is usually restricted to top soil layers (1–3 cm), and the same layers are enriched with fungitoxic residues after spraying the soil and leaching by irrigation. Benomyl concentration in the spray, and rate of irrigation, should be adjusted to each specific soil type for obtaining the desired effective concentration of the fungicide in the upper soil layer.

Extending the persistence of benomyl (or other pesticides) can also be achieved by other fungicides and by drastic biocidal means, e.g., fumigation and solarization (2,4,11); these are additional options to be studied. Pesticides frequently exert inhibitory or stimulatory effects on nontarget organisms. These may include pesticide degraders; thus, the fate of benomyl in soil may also depend on the pest management scheme employed for the crop.

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