Evaluation of Chemicals and Application Methods for Control of Bacterial Wilt of Tomato Transplants

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ABSTRACT

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Chloropicrin, whether covered with a polyethylene film or sealed with water, was the only soil treatment chemical of eight evaluated that provided significant full-season control of *Pseudomonas solanacearum* which causes bacterial wilt of tomato. A methyl bromide-chloropicrin mixture (67–33%) and DD-MENCS (a mixture of methyl isothiocyanate, dichlopropane and dichloropropene) retarded wilt development less effectively than chloropicrin but more effectively than methyl bromide. Methyl bromide gave good control only until midseason. Metham gave poor to moderate early-season control and was more effective when injected than when applied as a drench or incorporated. Potassium N-hydroxymethyl-N-methyldithiocarbamate and sodium azide provided limited early-season control. Hexachlorophene and formaldehyde were ineffective. Chloropicrin and methyl bromide applied at one-half, full, or twice the recommended rates (chloropicrin 326 L/ha, and methyl bromide 490 kg/ha) under optimum conditions, were highly effective in reducing high populations of *P. solanacearum* in artificially infested soils in greenhouse tests. Results of field and greenhouse experiments indicated that: high populations of *P. solanacearum* are necessary for bacterial wilt development, low residual pathogen populations remaining after chemical treatment rapidly increase to disease threshold levels in the presence of tomato roots, and chloropicrin is the most promising chemical for reducing bacterial populations in fields used for transplant production.

Additional key words: bacterial plant pathogen, *Lycopersicon esculentum, Pseudomonas solanacearum*, soil fumigation.

Approximately 700 million tomato seedlings are produced annually on about 4,000 acres in southern Georgia for transplanting to fruit producing areas of the northern United States and Canada (3,17). Infection by *Pseudomonas solanacearum* E. F. Sm., the cause of bacterial wilt, is a major cause for rejection of transplants (1,17). Furthermore, transplants with incipient infection may not be detected by field inspectors and may succumb to the wilt disease when transplanted into northern production fields (4,7,10,13). Presently, there is no effective control for bacterial wilt of tomato transplants except to avoid planting in areas infested with *P. solanacearum*.

Some general-purpose fumigants effectively reduce losses caused by soilborne fungi and nematodes and increase plant vigor and yields of marketable transplants (15,16). However, little information is available on the efficacy of these fumigants for controlling bacterial wilt. The present study was designed to evaluate various chemicals and methods of application for eliminating *P. solanacearum* from heavily infested soils, and to determine the rate of wilt development in a soil artificially infested with different levels of *P. solanacearum* in the greenhouse.

MATERIALS AND METHODS

Experiments were conducted in 1976 at Midville, in the middle coastal plain of Georgia on a Marlboro loamy sand, and in 1977 near Tifton, in the lower coastal plain on a Goldsboro loamy sand. The plot areas were infested previously by clipping thickly-seeded tomato transplants with a modified rotary mower contaminated with *P. solanacearum* followed by incorporation of the diseased plants into the soil (11). At Midville, the soil was heavily infested when treated in 1976; over 98% of the susceptible tomato plants grown in the plot area during the previous year became infected (12). The plot area at Tifton was artificially infested in 1975 and 1976 and was considered heavily infested by 1977. After land preparation in April or May of each year, the plots received a complete fertilizer mixture and the growing plants were sidedressed with calcium nitrate as needed. Plot beds (1.98 × 12.2 m at Midville and 1.8 × 15.2 m at Tifton) were prepared prior to chemical application each year.

Treatments at Midville were: methyl bromide (Dowfume MC-2 [Dow Chemical Co., Midland, MI 48604]), 490 kg/ha, applied with hand applicators under a 102-μm (4-mil) black polyethylene film; chloropicrin, 326 L/ha, injected only or injected and covered with polyethylene; DD-MENCS (20% methyl isothiocyanate + 80% 1,2-dichloropropane, 1,3-dichloropropene, and related chlorinated hydrocarbons), 280 L/ha, injected and covered with polyethylene; metham (sodium methyl dithiocarbamate), 748 L/ha, applied as a drench in 6,600 L of water, incorporated, or injected and covered with polyethylene film; PMDC (potassium N-hydroxymethyl-N-methyldithiocarbamate), 374 L/ha, incorporated; hexachlorophene (2,2'-methylenebis [3,4,6-trichlorophenol]), 0.9 L/ha, applied as a drench in 6,600 L of water; and control (no chemical treatment).

Treatments at Tifton were: methyl bromide, applied as at Midville; chloropicrin, and DD-MENCS, both at the same rates as used at Midville and injected without a cover or injected and covered with polyethylene; metham, applied as at Midville, and injected without a cover; PMDC, 748 L/ha, injected or injected and covered with polyethylene; MB-C (67% methyl bromide + 33% chloropicrin), 393 kg/ha, injected or injected and covered with polyethylene; sodium azide (NaN_3 granular), 58 kg a.i./ha, incorporated into the top 15 cm; formaldehyde (37%), 4,000 L/ha, applied as a drench, sealed immediately with 1.3 cm of water by sprinkler irrigation, and covered with polyethylene; and control (no chemical treatment).

At Midville, chemicals were injected 15–20 cm deep on 24-cm centers with a hand-operated Fumigun (Namco, Milpitas, CA 95035). At Tifton, chemicals were injected 15–20 cm deep with a

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tractor-mounted fumigator with injection chisels spaced 20 cm apart and equipped to reshape the beds and firm the soil during injection of the chemicals. At both Midville and Tifton, chemicals that were incorporated were applied to the soil surface in approximately 6,600 L of water and mixed into the top 15 cm of soil with a rotary tiller. All chemicals except those applied under polyethylene covers were sealed into the soil with 1.3 cm of water applied with a sprinkler irrigation system immediately after chemical application. Formaldehyde solution was drenched into the soil before the polyethylene cover was applied. Polyethylene covers were removed 2–5 days after chemical application.

In all experiments, treatments were arranged in a randomized complete block design with four replicated plots, each consisting of a double row of plants with 25 plants in each row.

Seven-week-old container-grown Marion tomato (*Lycopersicon esculentum* Mill.) plants were transplanted in the field plots in early June and at both sites and during both years were irrigated as needed to maintain vigorous growth. Foliar insects and diseases were controlled by applying recommended pesticides on a 7- to 10-day schedule.

Disease development and mortality were monitored throughout the growing season beginning when the first plants showed symptoms of bacterial wilt. At 3- to 7-day intervals, plants with symptoms were cut at soil level, and a section from each stem was checked for bacterial streaming in water (7). Suspensions from turbid tubes were streaked onto plates of triphenyl tetrazolium chloride agar (TCA) (8) and incubated at 35 C to confirm the presence of the wilt organism. At the end of the growing season all surviving plants were clipped at soil level and checked (by bacterial streaming from cut stem sections) for the presence of the bacterium.

Experiments were conducted in temperature tanks in the greenhouse to determine: the influence of soil inoculum level on the rate of disease development and the efficacy of chloropicrin and methyl bromide for reducing high levels of *P. solanacearum* in an artificially infested soil under optimum conditions for chemical activity. The soil used in all greenhouse studies was a Marlboro loamy sand which was steamed for two 3-hr periods on successive days.

Inoculum was prepared by streaking *P. solanacearum* onto plates of TCA, incubating them for 48–72 hr at 35 C, and selecting single virulent colonies for inoculum increase on plates of potato dextrose agar. The PDA plates were incubated for 48–72 hr at 35 C, and the bacteria were washed from the plates and suspended in sterile distilled water. Bacterial cells were sprayed onto batches of the soil during rotation in a cement mixer. In all tests, plants were grown in 3.8-L metal cans in temperature tanks maintained at 20–25 C during the first 2–3 days after transplanting and at 30–34 C thereafter.

Suspensions of the bacterium or sterile distilled water (the latter for the nontreated controls) were mixed with soil to give inoculum levels of 0, 2.5×10^4, 2.5×10^5, 2.5×10^6, 2.5×10^7, and 2.5×10^{10} cells per gram of air dry soil. Eighteen 6-wk-old, bare-rooted, container-grown Marion tomato plants were transplanted separately in cans containing each soil mix. The rate of disease development was recorded by noting the day on which wilt symptoms first appeared. Sections of stems from wilting plants were placed in water and the resulting suspensions were streaked onto plates of TCA to verify the presence of *P. solanacearum*. Plants that did not wilt were grown until 35–50 cm tall, cut at soil level, and each can was replanted with a young Marion seedling. This process was repeated when the first replants did not develop wilt symptoms. The study was continued for 180 days during which three tomato plants were grown in succession in some cans. A second inoculum-level study was conducted similarly except that the inoculum levels were 2.5×10^2, 2.5×10^3, 2.5×10^4, and 2.5×10^5 cells per gram of air dry soil and 13 6-wk-old bare-rooted Marion plants were transplanted into each soil mix. Pots were maintained until the plants wilted or for 150 days. Plants in both studies were fertilized with a water-soluble fertilizer solution as needed.

To test the chemical treatments, stream-sterilized soil, infested uniformly with 2.5×10^6 cells of *P. solanacearum* per gram was treated at rates comparable to 163, 326, or 652 L/ha of chloropicrin and 245, 490, or 980 kg/ha of methyl bromide representing one-half, full, and twice the recommended rates, respectively, of the two chemicals. Infested soil was placed in 76-L plastic garbage cans equipped with a 5-cm-diameter perforated hollow plastic pipe placed vertically in the center of the soil to enhance gas distribution. The cans were sealed with 102-μm (4-mil) polyethylene film, the chemical was metered into the center tube, the injection holes were plugged, and the container lid was placed on tightly. Cans were placed in a growth chamber operated at 28–30 C and were aerated after 2 days and 5 days for the methyl bromide and chloropicrin treatments, respectively. Ten days after treatment, the fumigated soils were mixed thoroughly to provide additional aeration and placed into 3.8-L cans (20 per fumigant). A single 6-wk-old Big Boy tomato plant was bare-root transplanted in each can, and the cans were placed in temperature tanks. Disease development was recorded daily after the first disease symptoms appeared. Plants that had not wilted 34 days after transplanting were cut at soil level and replaced with Marion seedlings as described previously. The process was repeated when plants did not wilt after the second replanting. The study was continued for 152 days with three plants grown in succession in some cans.

RESULTS

The effectiveness of the chemical treatments in controlling bacterial wilt varied greatly at both Midville in 1976 (Table 1) and Tifton in 1977 (Table 2). No treatment provided complete control at either location; several treatments provided good early-season control, but even with the best, a high percentage of the plants had died by the end of the growing season. In both experiments, chloropicrin applied by either method was the most effective of the eight chemicals evaluated. It was also the only chemical that significantly altered final plant survival counts at Midville. The DD-MENCS and MB-C treatments were about equally effective and both provided good early-season control. However, a high percentage of plants eventually became infected. Methyl bromide also gave good control at both locations until mid- to late-season when plants began to wilt; 90% mortality was reached by the end of the season. Metham was rated poor to moderate in effectiveness in retarding wilt, depending on the application method. Generally, metham was more effective when injected, either with or without a cover. More than 90% of plants in plots treated with metham as a drench or incorporated were killed by the wilt organism by the end of the season. The PMDC treatment at 374 L/ha did not significantly alter the rate of disease development or final survival at Midville; at 748 L/ha, the chemical did provide some early-season control at Tifton. Results were similar with sodium azide, but the period of control was even shorter. Neither hexachlorophene nor formaldehyde retarded disease development.

The rate of bacterial wilt development varied greatly with inoculum level in steam-sterilized soil in the greenhouse. In the first study, plants transplanted into soil with inoculum levels of 2.5×10^7 and 2.5×10^{10} cells per gram wilted initially in an average of 4–5 days, and all plants wilted within 10 days after transplanting. All plants growing in soil with 2.5×10^6 cells per gram also were killed rapidly; symptoms appeared in 4–23 days (7.6 avg). No other inoculum level resulted in complete kill of plants in either study, although a minimum of 31% eventually were killed at each inoculum level. The number of plants killed and the speed of disease development generally increased with inoculum level. Frequently, two plants transplanted successively into soil with the low inoculum levels remained healthy, and wilt symptoms did not appear until the third plant was transplanted into the same can. An inoculum level of 2.5×10^6 was considered appropriate for the chemical eradication studies because disease developed normally at this level, and all plants were killed.

In the greenhouse, some plants growing in nonfumigated soil showed initial symptoms in 8 days, and all 20 had wilted within 19 days (12.2 days avg time). Plants growing in infested soil treated with chloropicrin or methyl bromide, regardless of the rate, remained mostly free of bacterial wilt during the first 80 days of the study. None of the first plants grown in the treated soils showed symptoms within the first 34 days. Only one plant (from soil treated

with one-half the recommended rate of methyl bromide) had bacteria in its vascular system after 34 days. Two additional plants (one from one-half the recommended rate of chloropicrin and one from the full recommended rate of methyl bromide) wilted when a second plant was transplanted into each container. These plants wilted late (50 and 64 days after transplanting), which indicated a low level of infestation. When the plants were cut and a seedling was transplanted to the soil a second time after 80 days, nearly all (95–100%) of the plants growing in soil treated with chloropicrin or methyl bromide, regardless of rate, remained free both of wilt symptoms and of bacteria in their vascular systems. However, the disease developed slowly in additional plants growing in soil treated at one-half the recommended and the full recommended rates of methyl bromide during the 80–152 day period. At the end of the study, 90, 55, and 0%, and 15, 0, and 0% plants growing in soil treated one-half, the full recommended, and twice the recommended rates of methyl bromide and chloropicrin, respectively, either had died of wilt or had high populations of bacteria in their vascular systems.

DISCUSSION

Tomato transplant growers in southern Georgia are interested in determining the most effective chemicals and methods of application for eliminating or controlling soilborne bacterial and fungal plant pathogens from permanent production sites (15–17). The results of our field tests indicate that chloropicrin, even when applied without a cover, may be the most effective general-purpose fumigant for controlling *P. solanacearum*. Fall fumigation may be necessary since tomato transplants are seeded early in the spring, and chloropicrin dissipates slowly from the soil at low temperatures. The fumigants MB-C, DD-MENCS, and to some extent methyl bromide, also should provide some control; they provided early-season control in our studies. Tomato transplant crops are seeded when conditions are less optimum for bacterial wilt development than in our experiments, and the plants reach marketable size in about 8 wk. The metham treatments generally were less effective than chloropicrin, MB-C, DD-MENCS, or methyl bromide, and effectiveness depended on method of application. The injection treatments probably were more effective because the chemical penetrated deeper. At economical rates, PMDC and sodium azide did not control bacterial wilt of tomato. Hexachlorophene and formaldehyde were ineffective in our experiments, although formalin reduced the severity of bacterial wilt in earlier studies (7,19). In earlier work (7), chloropicrin treatment also reduced severity of Granville wilt of tobacco (also caused by *P. solanacearum*), but it did not eliminate the bacterium from the soil. DD-MENCS applied under polyethylene film reduced the severity of bacterial wilt of tomato in Florida but provided no control when injected without a

TABLE 1. Effectiveness of various chemical treatments of soil for the control of bacterial wilt on tomato plants at Midville, GA, during 1976

Treatment[a]	Percentage of plants with bacterial wilt[b] after transplanting plus: 47 days	58 days	67 days	78 days	88 days	89[c] days
Methyl bromide	1.0 v[d]	15.0 xy	27.5 x	63.0 z	89.0 z	90.0 z
Chloropicrin, injected	1.0 v	8.5 x	12.0 x	26.5 y	61.0 y	64.5 y
Chloropicrin, injected, covered	8.0 vw	13.5 x	15.0 x	18.5 y	58.5 y	60.0 y
DD-MENCS, injected, covered	4.5 vw	11.5 x	18.0 x	36.0 y	90.5 z	92.5 z
Metham, drench	21.0 xyz	52.0 z	61.0 yz	78.5 z	93.5 z	93.5 z
Metham, incorporated	13.5 xy	49.5 z	60.0 yz	87.5 z	94.0 z	95.0 z
Metham, injected, covered	11.5 wx	31.5 y	43.5 xy	73.0 z	95.0 z	95.0 z
PMDC, incorporated	31.0 z	62.5 z	69.5 z	81.0 z	86.5 z	86.5 z
Hexachlorophene, drench	22.0 yz	65.5 z	74.5 z	87.5 z	87.5 z	87.5 z
Control (no chemical)	23.5 yz	65.5 z	75.0 z	87.0 z	87.5 z	88.0 z

[a]Details on rates and methods of application are given in the text. DD-MENCS = mixture of methyl isothiocyanate, dichloropropanes, and dichloropropene. PMDC = potassium N-hydroxymethyl-N-methyldithiocarbamate.
[b]Each value is a mean of four replications of 50 plants each.
[c]Values for 89 days include plants that had the wilt bacterium in the vascular system at the end of the experiment but had not wilted.
[d]Values followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

TABLE 2. Effectiveness of various soil chemical treatments for the control of bacterial wilt of tomato at Tifton, GA, during 1977

Treatment[a]	Percentage of plants with bacterial wilt[b] after transplanting plus: 34 days	47 days	57 days	67 days	74 days	81 days	91 days	102 days	116[c] days
Methyl bromide	1.5 w[d]	5.0 vw	10.5 uvw	20.0 wx	24.5 wx	40.0 wxy	57.0 v-z	83.5 z	93.0 yz
Chloropicrin, injected	0.0 w	0.0 v	0.5 u	0.5 w	0.5 w	8.0 vw	15.0 tu	27.5 vw	55.0 wx
Chloropicrin, injected, covered	0.0 w	0.0 v	0.0 u	1.0 w	1.0 w	3.0 v	8.0 t	18.5 v	51.5 w
DD-MENCS, injected	0.0 w	1.0 v	1.0 u	2.0 w	4.0 w	9.0 vw	16.0 tu	36.0 vwx	70.0 w-z
DD-MENCS, injected, covered	0.0 w	0.5 v	1.0 u	1.5 w	3.5 w	14.0 vw	34.0 t-x	55.5 w-z	72.5 w-z
Metham, drench	4.5 wy	15.5 xy	29.0 xy	51.0 yz	58.5 yz	73.0 yz	82.5 yz	91.0 z	96.0 z
Metham, incorporated	10.5 x	22.5 y	41.5 z	61.5 yz	64.5 yz	77.0 z	82.5 yz	87.0 z	90.5 yz
Metham, injected	0.5 w	4.5 vw	7.5 uv	18.5 wx	24.5 wx	37.0 wx	48.5 u-z	56.0 w-z	70.0 w-z
Metham, injected, covered	0.5 w	5.0 vw	9.0 uvw	14.5 wx	20.0 wx	32.0 vwx	39.0 tuv	55.0 w-z	67.5 w-z
PMDC, injected	1.5 w	3.0 v	6.5 uv	18.5 wx	26.0 wx	37.0 wx	57.5 v-z	70.5 xyz	88.5 yz
PMDC, injected, covered	1.0 w	2.0 v	3.0 u	18.0 wx	19.0 wx	32.0 vwx	47.5 u-y	64.0 w-z	78.5 w-z
Methyl bromide-chloropicrin (67-33), injected	0.5 w	0.5 v	1.5 u	5.0 w	5.5 w	12.5 vw	22.0 tuv	39.5 vwx	76.5 w-z
Methyl bromide-chloropicrin (67-33), injected, covered	0.0 w	0.0 w	0.5 u	1.5 w	3.5 w	14.0 vw	28.5 t-w	45.0 v-y	71.0 wxy
Sodium azide, incorporated	2.5 w	12.0 vwy	24.5 vwx	38.5 xy	47.0 xy	60.0 xyz	68.5 w-z	74.0 xyz	81.0 xyz
Formaldehyde, drench, covered	23.5 y	44.5 z	56.0 z	74.0 z	75.5 z	83.0 z	84.5 z	84.5 z	85.0 yz
Control (no chemical)	15.5 z	29.0 xy	34.5 wxy	52.5 xy	62.5 xy	74.0 xyz	83.5 xyz	85.5 yz	86.0 yz

[a]Details on the rates and methods of application are given in the text. DD-MENCS = mixture of methyl isothiocyanate, dichloropropanes, and dichloropropene. PMDC = potassium N-hydroxymethyl-N-methyldithiocarbamate.
[b]Each value is a mean of four replications of 50 plants each.
[c]Values for 116 days include plants that had the wilt bacterium in the vascular system but had not wilted.
[d]Values followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

cover (6).

The greenhouse experiment to test different rates of chloropicrin and methyl bromide allowed a comparison of the chemicals under optimum conditions. Both fumigants, even when applied at one-half the recommended rates, effectively reduced a heavy infestation of *P. solanacearum* in artificially infested soil although at the full recommended rate chloropicrin was more effective than methyl bromide. Chloropicrin at the full recommended and double the recommended rates apparently eliminated the bacterium from a heavily infested soil. The slow development of wilt in plants growing in soil treated with one-half the recommended rate of chloropicrin indicated a buildup of the bacterium in the presence of tomato roots from a low residual population of the pathogen.

Probable explanations for the relatively poor performances of chloropicrin and methyl bromide in the field compared with those in the greenhouse are: suboptimum moisture or structural conditions for fumigant action at certain sites in the soil environment; spread of the bacterium by root-to-root contact; the presence of bacterial cells deeper in the profile than fumigant penetration; and some reinfestation of treated areas as the season progressed. Fumigants applied by the methods used in our tests probably would not penetrate in lethal concentrations deeper than 30 cm, and *P. solanacearum* has been found at depths of 60–75 cm (2,14). Since some reinfestation probably occurred in our test, the chemicals should be more effective when applied broadcast, which would be likely in tomato transplant production.

Our greenhouse studies support earlier findings (5,9,14) that relatively high levels of inoculum in the rhizosphere are required for bacterial wilt development. Inoculum levels of 2.5×10^6 cells per gram of soil provided rapid disease development under our conditions. The inoculum potential required for disease development may vary with the soil involved because recent work (18) suggested that biological factors may suppress *P. solanacearum* in some soils, and these factors also could influence the rate of disease development at a given inoculum level.

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