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## REINVASION OF METHYL BROMIDE TREATED SOIL BY SOIL-BORNE FUNGI AND THEIR SUBSEQUENT EFFECT ON CITRUS SEEDLING GROWTH<sup>1,2</sup>

W. H. RIDINGS

*Florida Department of Agriculture and Consumer Services,  
Division of Plant Industry,  
Gainesville, FL 32602*

N. C. SCHENCK

*University of Florida,  
Institute of Food and Agricultural Sciences,  
Department of Plant Pathology,  
Gainesville, FL 32611*

R. R. SNELL AND W. M. KEEN

*Florida Department of Agriculture and Consumer Services,  
Division of Plant Industry,  
Winter Haven, FL 33880*

J. A. CORNELL

*University of Florida,  
Institute of Food and Agricultural Sciences,  
Department of Statistics,  
Gainesville, FL 32611*

**with the high degree of root rot. *Fusarium oxysporum* and *F. solani*, although isolated from roots of diseased citrus plants, were not closely associated ( $r^2 = 0.04$ ) with the root rot problem.**

The Premium Quality Citrus Nursery Tree Program of the Florida Department of Agriculture and Consumer Services was designed to provide the citrus industry with high quality, disease-free trees produced in citrus nurseries. Since the initiation of this volunteer program in 1969-70, several problems have developed in some nurseries of which a serious root rot loss of citrus seedlings in soil fumigated with  $\text{CH}_3\text{Br}$  has been the most widespread.

In 1975, investigations were initiated into possible explanations for the root rot problems in citrus nurseries. This paper reports the results of fumigation with different rates of  $\text{CH}_3\text{Br}$  (as Brozone), reinvasion by different soil fungi, and the subsequent growth of citrus seedlings in one nursery.

### Materials and Methods

A citrus nursery in central Florida was chosen for the test site. This nursery was one of several that was experiencing a severe root rot problem with citrus seedlings grown in  $\text{CH}_3\text{Br}$  (as Brozone) treated soil.

The selected test area was 500 x 50 ft (152.4 x 15.2 m) and had not been cultivated or fumigated for 2½-3 years. This test area was treated with dolomite at 3136 lbs/acre (3515 kg/ha) and triple superphosphate at 1045.3 lbs/acre (1171.6 kg/ha) prior to dividing into two test areas which were further subdivided for the fumigation treatments. Test area A consisted of 12 plots each of which was 10 x 100 ft (3.0 x 30.5 m) and which were arranged in 3 randomized complete blocks of 4 randomized plots each. Test area B consisted of 16 plots each of which was 17 x 15 ft (5.2 x 4.6 m) and which were arranged in 4 randomized complete blocks of 4 randomized plots each. The area of the actual seed planting was 5 x 2.5 ft (1.5 x 0.75m) in the center of each plot.

In test area A, Brozone (68.6%  $\text{CH}_3\text{Br}$ , 1.4% chloropicrin, 30.0% inert petroleum) was released through tractor-drawn chisels at 6 in (15 cm) spacing at soil depths of 6-8 in (15-20.3 cm) over a 10 x 100 ft (3.0 x 30.5 m) spacing. The fumigated areas were covered immediately with 1¼ mil (0.03 mm) polyethylene tarp. The amount of gas released was determined by calibration of the tractor speed and weight of Brozone gas released over a given distance. The fumigation rates were at 0, 1, 2, and 3 lbs (0.45, 0.90, and 1.36 kg)  $\text{CH}_3\text{Br}$  (as Brozone) per 100 sq. ft. (9.29 m<sup>2</sup>). Fumigation of plots in test area B was done by releasing

*Additional index words.* Mycorrhizae, nematodes, *Phytophthora*, *Fusarium*.

**Abstract.** Soil samples were taken from a citrus nursery prior to and following treatment of field plots with Brozone at 0, 1, 2, and 3 lbs (0, 0.45, 0.90, and 1.36 kg) of methyl bromide per 100 sq. ft. (9.29 m<sup>2</sup>). Soil was sampled from the first 6 in (15 cm) and from 18-24 in (45-60 cm) at 0, 2, 4, 6, 9, and 12 months after fumigation and assayed for *Phytophthora*, *Pythium*, *Fusarium*, vesicular-arbuscular (VA) mycorrhizal fungi, and other soil-borne fungi. Carrizo seedlings were sampled at 2, 4, 6, 9 and 12 months after fumigation and assayed for root infection by *Phytophthora*, *Pythium*, *Fusarium*, and VA mycorrhizae. Data on root rot, tap root length, stem length, and total plant weight were recorded. All rates of fumigation gave effective reduction of *Phytophthora*, *Pythium*, *Fusarium*, and VA mycorrhizal fungi. After fumigation *Fusarium* spp. were detected in the soil within 2 months, *Phytophthora* and *Pythium* within 4 months, and VA mycorrhizal fungi within 2 to 12 months. Regression analyses showed that early recovery of *Phytophthora parasitica* from the roots was most closely associated ( $r^2 = 0.56$ )

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predetermined amounts of Brozone at 0, 1, 2, and 3 lbs (0, 0.45, 0.90, and 1.36 kg) of  $\text{CH}_2\text{Br}/100$  sq. ft. (9.29  $\text{m}^2$ ) under a 4 mil (0.10 mm) polyethylene tarp covering 17 x 15 ft (5.2 x 4.6 m). The determined weight of gas for the plot was released by following the weight loss from a 125 lb (56.7 kg) Brozone-filled cylinder into a reservoir in the center of the plot. Fumigation of both test areas was conducted on June 20, 1975. The soil temperature at the time of fumigation was 29-31 C within the first 6 in (15 cm), and soil moisture ranged from 5.3 to 5.6% as determined by the weight loss in oven dried soil.

Tarps were removed from the plots 10 days after fumigation, and Carrizo seeds were hand-planted approximately 2-3 weeks later on July 23 and 24, 1975. Over 90 percent of the Carrizo seed germinated by August 25, 1975. Seeds were planted at one-inch (2.5 cm) spacing with 6-in (15 cm) spacing between the rows. Seeds for the test were obtained from Lake Unity Nursery in Leesburg, Florida.

Fertilizer was applied weekly through the irrigation system by the nursery owner using  $\text{NH}_4\text{NO}_3$  at the rate of 1000 gal (3780 liter) of 8% N over 40 acres (16.2 ha). Additional fertilizer was applied on February 12, 1976, and April 29, 1976 with the formulations 6-6-6 and 8-8-8 at the rate of 1 lb (0.45 kg) of each fertilizer for each seed plot size of 5 x 2 $\frac{1}{2}$  ft (1.5 x 0.75 m) at each date. The 6-6-6 was an organic fertilizer ( $\text{H}_2\text{O}$  soluble N = 2.5%;  $\text{H}_2\text{O}$  insoluble N = 3.5%) which also contained 2.0% MgO, 0.1% MnO, 0.04%  $\text{CuSO}_4$ , 0.1%  $\text{B}_2\text{O}_3$ , 1.0%  $\text{Fe}_2\text{O}_3$ , and 0.003%  $\text{MoO}_2$ . The 8-8-8 was an inorganic fertilizer (nitrate N = 2.5%; ammoniacal N = 5.5%) which also contained 0.097%  $\text{B}_2\text{O}_3$ , 0.0375% CuO, 1.0%  $\text{Fe}_2\text{O}_3$ , 0.095% MnO, 0.003%  $\text{MoO}_3$ , and 0.0875% ZnO. Other nutrients supplied to the seedlings were microelements contained in Perk (7.5% Cu, 51.0% S, 7.0% Zn, and 5.5% Mn) applied at the rate of 35 g/2 gal (7.6 liter) with the fungicide sprays.

Seedlings were treated with protective fungicides effective against scab (*Elsinoe fawcetti* Bitanc. & Jenk.) beginning February 24 and ending May 14, 1976. The fungicides employed included Difolatan 4F at 1 gal (3.78 liter) per 100 gal (378 liter) water and Kocide 101 at 1.5 lb (0.68 kg) per 100 gallons (378 liter) water. These fungicides plus a spreader-sticker (Plyac) were applied on an alternating 2-week spray schedule. Perk, a micronutrient supplement was applied with the Kocide 101 applications.

General maintenance of the plots included hand weeding of the seedlings and scuffle hoeing the remainder of the plots. The areas between the plots were hoed and periodically treated with herbicides.

Soil samples were taken within the first 6 in (15 cm) and 18-24 in (45-60 cm) at 0, 2, 4, 6, 9, and 12 months after seed planting. Twelve separate soil core samples were drawn at random from the first 6 in (15 cm) in the area of the seedlings. In addition, five of the 12 initial holes were sampled at the 18-24 in (45-60 cm) depth. The sample tool was surface-disinfested in 0.525% sodium hypochlorite for approximately 5 minutes and rinsed between plots. Additional care was taken to avoid unnecessary contamination of the plots by placing plastic bags over one's feet each time a plot was entered. These soil samples were subdivided and assayed for the presence of *Phytophthora*, *Pythium*, *Fusarium*, vesicular-arbuscular (VA) mycorrhizal fungi, other soil-borne fungi, and nematodes. *Phytophthora* and *Pythium* spp. were detected in the soil samples by the citrus leaf piece baiting technique (5) and by direct plating of 1 g samples of soil on a selective medium of cornmeal agar (Difco) containing 10 ppm pimaricin, 200 ppm vancomycin, and 100 ppm pentachloronitrobenzine (CMA-PVP) (15). *Fusarium* spp. were detected by soil dilution (0.3 g/100 ml

of 0.1% water agar) and plating on a selective medium for *Fusarium* spp. (11). VA mycorrhizal fungi were recovered by planting soybean seeds in the soil samples and allowing 90 days before assaying the roots for infection. The total soil-borne fungi were determined by placing 1 ml samples of a soil suspension (1 g in 3000 ml) into melted and cooled potato dextrose agar containing Tergitol NPX (13). The plates for total fungi and *Fusarium* spp. were incubated under continuous fluorescent light (Westinghouse C/W F15T8, 125-250 ft-c) for 5-7 days before colony counts were recorded. Five to 10 plates were used for each soil sample. The soil pH was recorded for each sample, and a general soil analysis was conducted for composite samples at the beginning and end of the investigation.

Seedlings were sampled at random from each plot on the same dates as the soil samples. Data recorded for the seedlings included stem length, tap root length, total plant weight, VA mycorrhizal infection, and a root rot rating (1 = healthy; 2 = less than 10% of roots rotted; 3 = 10-25% of roots rotted; 4 = 26-75% of roots rotted; 5 = 76-90% of roots rotted; and 6 = more than 90% of roots rotted). Also, roots were excised from the plants and surface-treated with 0.11% sodium hypochlorite for 4 minutes, rinsed in sterile tap water, blotted, and plated on acidified potato dextrose agar and on the selective medium CMA-PVP.

## Results

Soil samples taken immediately following removal of the tarps from the plots with the high rates (2 and 3 lb) (0.91 kg and 1.36 kg) yielded no *Phytophthora*, *Pythium*, *Fusarium*, or VA mycorrhizal fungi at either soil depth in both test sites (Table 1 and 2). Soil samples from the one pound (0.45 kg) rate of methyl bromide yielded *Pythium* in one plot and *Fusarium* in 2 plots of test area A. *Fusarium* was recovered from only one plot in test area B. No *Phytophthora* or VA mycorrhizal fungi were detected in any fumigated plot from either test area. The nonfumigated plots (controls) in test area B showed a more extensive soil infestation with *Phytophthora*, *Pythium*, and *Fusarium* than in test area A. All control plots showed the presence of VA mycorrhizal fungi in the first 6 in (15 cm) of soil. In general, the soil samples from the first 6 in (15 cm) of the control plots revealed a more extensive infestation with fungi than those soil samples from the 18-24 in (45-60 cm) depth. Counts of total fungi in the first 6 in (15 cm) after fumigation at all rates were lower than those in nonfumigated plots for both test areas (Table 3). No reductions in total count occurred at the 18-24 in (45-60 cm) depth. The fungi which were included in the counts consisted primarily of species of *Penicillium*, *Trichoderma*, *Alternaria*, *Fusarium*, *Rhizopus*, *Aspergillus*, *Curvularia*, *Cladosporium*, *Helminthosporium*, *Diplodia*, *Pestalotia*, *Coniothyrium*, *Gloeosporium* and *Chalaropsis*.

The earliest detection of *Phytophthora* in the soil of fumigated plots at the first 6 in (15 cm) level varied from 4 months to 12 months after planting, and the first detection of *Pythium* varied from planting time to 6 months later (Table 1). *Fusarium* was detected from 2 to 6 months after planting and the VA mycorrhizal fungi from 2 to 12 months after planting (Table 2).

The early recovery of fungi from the different plots was highly variable and not consistent with a given rate of fumigation. In general, test area B proved more suitable than test area A for the early recovery of *Phytophthora* and VA mycorrhizal fungi from the soil. There did not appear to be any major difference in the recovery time (sample date of first detection) for the *Fusarium* and *Pythium* between

Table 1. Earliest detection of *Phytophthora* and *Pythium* in soil and roots of Carrizo seedlings from two test areas.

Test area	Fumigation rate*	Plot no.	Sample time <sup>†</sup> for detection of fungi at two soil depths and in roots					
			<i>Phytophthora</i>			<i>Pythium</i>		
			0-15 cm	45-60 cm	roots	0-15 cm	45-60 cm	roots
A	0	1	6	6	4	6	6	4
		2	1	0	1	5	5	3
		3	2	0	2	6	5	0
	1	1	1	0	1	3	2	0
		2	1	0	1	6	3	0
		3	1	0	0	3	3	0
	2	1	1	0	0	3	0	1
		2	1	0	1	3	3	0
		3	0	0	0	3	0	0
	3	1	2	0	2	4	2	0
		2	0	0	0	3	0	0
		3	1	0	1	3	3	0
B	0	1	6	6	4	6	6	3
		2	1	0	1	6	6	0
		3	6	4	4	6	4	3
		4	6	4	4	6	6	3
	1	1	4	4	4	4	4	0
		2	1	0	2	3	3	2
		3	3	2	3	3	3	0
		4	1	0	1	4	4	0
	2	1	0	0	0	3	3	0
		2	2	0	2	3	0	0
		3	3	0	1	4	3	0
		4	3	0	1	4	0	0
3	1	2	0	2	4	3	0	
	2	2	0	2	4	0	0	
	3	1	0	1	4	3	0	
	4	4	4	2	4	4	0	

Table 2. Earliest detection of *Fusarium* and VA mycorrhizae in soil and roots of Carrizo seedlings from two test areas.

Test area	Fumigation rate*	Plot no.	Sample time <sup>†</sup> for detection of fungi at two soil depths and in roots					
			<i>Fusarium</i>			VA mycorrhizae		
			0-15 cm	45-60 cm	roots	0-15 cm	roots	cm
A	0	1	6	6	5	6	5	
		2	5	6	5	6	5	
		3	6	5	5	4	3	
	1	1	6	5	2	1	1	
		2	6	5	1	2	2	
		3	5	5	1	2	2	
	2	1	3	5	1	2	2	
		2	5	5	2	1	1	
		3	5	5	2	0	0	
	3	1	2	5	2	2	1	
		2	5	5	1	0	0	
		3	2	5	5	3	0	
B	0	1	6	6	5	6	5	
		2	6	6	5	4	4	
		3	6	6	5	6	5	
		4	6	6	5	6	5	
	1	1	4	5	3	2	2	
		2	5	5	5	2	1	
		3	6	4	3	5	0	
		4	5	5	2	2	0	
	2	1	5	5	2	2	2	
		2	5	5	2	2	2	
		3	5	5	5	5	1	
		4	5	5	3	2	1	
3	1	3	5	3	2	2		
	2	5	5	2	2	1		
	3	5	5	5	2	2		
	4	5	5	3	3	2		

\*Pounds of methyl bromide (as Brozone) per 100 sq. ft.

<sup>†</sup>0 = not detected at any sample time  
 1 = detected at 12-month sample time  
 2 = " " 9-month " "  
 3 = " " 6-month " "  
 4 = " " 4-month " "  
 5 = " " 2-month " "  
 6 = " " planting

\*Pounds of methyl bromide (as Brozone) per 100 sq. ft.

<sup>†</sup>0 = not detected at any sample time  
 1 = detected at 12-month sample time  
 2 = " " 9-month " "  
 3 = " " 6-month " "  
 4 = " " 4-month " "  
 5 = " " 2-month " "  
 6 = " " planting

test areas. The detection of the fungi in soil at the 18-24 in (45-60 cm) depth was generally later than the recovery time for the first 6 in (15 cm). However, *Fusarium* spp. were detected in several plots initially at the deeper level (Table 2). In general, the recovery of *Phytophthora*, *Fusarium*, and VA mycorrhizae from the roots occurred within the same time or soon after detection in the soil. Very few root samples showed infection by *Pythium* spp. even though the fungus was in the soil 6-8 months before the termination of the investigation (Table 1). The identification of the *Phytophthora*, *Fusarium*, and VA mycorrhizae from the roots revealed *Phytophthora parasitica* Dast. (*P. nicotianae* B. de Haan var. *parasitica* (Dast.) Waterh.), *Fusarium oxysporum* Schlecht., *F. solani* (Mart.) Sacc., and *Glomus macrocarpus* Tul. & Tul.

Nematodes were recovered from the 18-24 in (45-60 cm) depth samples at various times during the experiment. A low population of *Helicotylenchus* sp. was detected from soil in one plot of the controls of test area A at 2 months after planting. After 4 months a low population of *Helicotylenchus* sp. and *Meloidogyne* sp. was detected in one additional control plot in test area A. After 6 months, low populations of *Helicotylenchus* sp., *Meloidogyne*, and *Criconemoides* were detected from 2 plots treated with 3 lb (1.36 kg) of CH<sub>3</sub>Br in test area B. However, no nematode damage was found on the seedlings in this test.

The soil pH at seed planting in test area A varied from

Table 3. Colony count of total fungi immediately following fumigation.

Test area	Fumigation rate	Colony counts x 10 <sup>3</sup> of total fungi	
		0-15 cm	45-60 cm
A	0	22.8	5.4
	1	13.8	6.3
	2	7.5	7.8
	3	4.8	4.5
B	0	15.0	6.3
	1	2.7	5.7
	2	5.7	4.2
	3	3.3	9.0

6.7 to 7.5 at the first 6 in (15 cm) level and from pH 6.8 to 7.0 at the 18-24 in (45-60 cm) level. After 12 months, the soil pH was 6.8 at the first 6 in (15 cm) level and pH 6.0 at the 18-24 in (45-60 cm) level. The soil pH at seed planting in test area B varied from pH 6.8 to 6.9 at the first 6 in (15 cm) level and pH 6.9 to 7.1 at the 18-24 in (45-60 cm) level. After 12 months, the soil pH was 7.0 at the first 6 in (15 cm) level and pH 6.3 at the 18-24 in (45-60 cm) level.

The soil analyses in the first 6 in (15 cm) after 12 months showed a greater amount of CaO in test area A

(1450 kg/ha) than in test area B (931 kg/ha). There was very little difference between the areas in the remaining nutrient levels of MgO ( $\bar{x}$  = 183 kg/ha), P<sub>2</sub>O<sub>5</sub> ( $\bar{x}$  = 64 kg/ha), and K<sub>2</sub>O ( $\bar{x}$  = 54 kg/ha).

The growth of the seedlings in all plots was checked at each soil sample time, and the results of the 12-month harvest are presented in Table 4. In general, seedling development with regard to stem length, tap root length, and total weight was greater in test area A than in test area B. This was especially evident in the control plots. The root rot rating was greater in test area B than in test area A for all plots (Fig. 1).

Table 4. Growth of Carrizo seedlings and root rot rating in two test areas after 12 months.

Test area	Fumigation rate <sup>2</sup>	Plot no.	Growth parameter and measurement* of growth of seedlings			
			Stem length cm	Tap root length cm	Total wt g	Root rot rating <sup>x</sup>
A	0	1	25.9	14.6	7.7	3.2
		2	47.7	26.9	20.2	2.2
		3	48.9	27.2	17.5	1.8
	1	1	38.4	25.5	13.5	1.6
		2	43.4	26.6	18.9	1.4
		3	57.7	36.1	21.2	1.0
	2	1	32.5	28.0	11.3	1.0
		2	50.0	31.2	18.9	1.7
		3	52.2	30.9	20.6	1.1
	3	1	44.9	25.9	18.1	1.7
		2	43.2	30.4	18.1	1.1
		3	39.7	26.5	15.1	2.0
B	0	1	32.9	17.2	10.6	3.2
		2	31.5	23.5	10.5	1.7
		3	21.1	9.3	8.2	4.4
		4	25.7	12.8	9.2	3.7
	1	1	16.8	11.0	6.2	3.7
		2	32.6	26.7	14.9	2.1
		3	36.1	27.3	15.5	1.9
		4	38.6	25.5	14.6	1.8
	2	1	35.8	28.9	14.6	1.4
		2	32.7	26.4	14.2	1.3
		3	33.6	25.2	13.7	2.4
		4	35.8	25.6	14.5	3.8
3	1	49.8	27.2	24.2	1.9	
	2	38.3	26.2	16.1	2.8	
	3	44.2	31.9	19.7	1.9	
	4	44.9	26.1	20.5	1.4	

\*Growth measurement based on 25 plants from each replicate plot.

<sup>2</sup>Pounds of actual methyl bromide (as Brozone) per 100 sq. ft.

<sup>x</sup>1 = healthy; 2 = less than 10% of roots rotted; 3 = 10-25% of roots rotted; 4 = 26-75% of roots rotted; 5 = 76-90% of roots rotted; 6 = more than 90% of roots rotted.

Correlation coefficients (12) were calculated between root rot rating and the seedling growth parameters of stem length ( $r = -0.68$ ), tap root length ( $r = -0.83$ ), and total plant weight ( $r = -0.63$ ). These correlations were highly significant ( $P < 0.01$ ). Recovery times for *P. parasitica*, *Pythium* spp., *Fusarium* spp. (*F. oxysporum* and *F. solani*), and *G. macrocarpus* were also correlated with root rot rating. The first three ( $r = 0.75$ ,  $r = 0.56$ ,  $r = 0.55$ ) were highly significant while the latter ( $r = 0.39$ ) was significant only at the 0.05 level.

A regression analysis was conducted with tap root length (TRL), root rot rating (RRR), and total plant weight (TPW) fitted each as the dependent variable and root recovery time (RRT) for *P. parasitica* (P) and *Fusarium* spp. (F) as the independent variables. The TRL regression

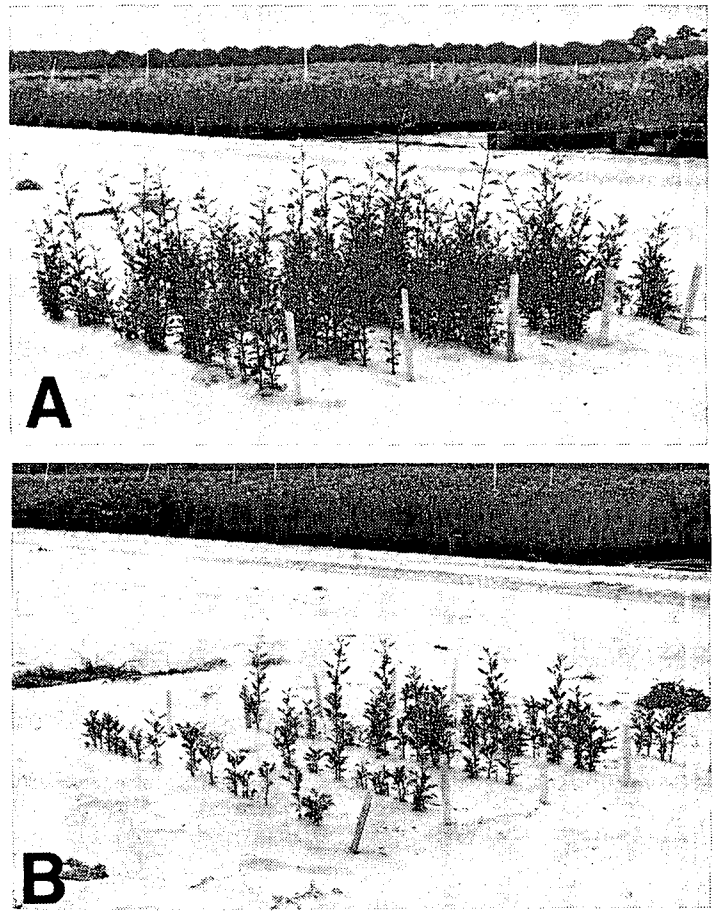


Fig. 1. Carrizo seedlings in plots having different recovery times of *Phytophthora parasitica* from the roots after 12 months: A) Late recovery time, B) Early recovery time.

equation was best as evidenced by its R<sup>2</sup> value of 0.79 when compared to RRR (R<sup>2</sup> = 0.60) and TPW (R<sup>2</sup> = 0.51). The empirical formula for predicting TRL was TRL = 30.3 + 1.20 P-RRT - 0.54 F-RRT - 1.17 (P-RRT)<sup>2</sup>.

With the simple regression equation TRL = 31.4 - 3.9 P-RRT, the R<sup>2</sup> value equalled 0.69, implying that almost 70 percent of the total variation in the TRL values was explained by the influence of RRT of *P. parasitica* only. Regressing TRL on F-RRT resulted in a value of R<sup>2</sup> = 0.23. A similar trend resulted when RRR was regressed on P-RRT and F-RRT separately. For *P. parasitica*, the equation was RRR = 1.27 + 0.51 P-RRT with an R<sup>2</sup> = 0.56. For *Fusarium* spp., the equation was RRR = 1.05 + 0.33 F-RRT with an R<sup>2</sup> = 0.31. When both P-RRT and F-RRT were fitted, the equation was RRR = 0.97 + 0.43 P-RRT + 0.136 F-RRT with R<sup>2</sup> = 0.60. The increase from 0.56 to 0.60 in the R<sup>2</sup> value represents the influence of *Fusarium* spp. after the effect of *P. parasitica* has been realized.

## Discussion

The effectiveness of CH<sub>3</sub>Br against plant pathogenic fungi and other pests has been well documented (1, 7). The results of this fumigation test agree with those obtained by other workers. Methyl bromide (as Brozone) was effective against *P. parasitica* at all rates employed, but *Pythium* spp. and *Fusarium* spp. survived the lower rates of fumigation.

The reinvasion of fumigated plots by soil-borne fungi appeared to be random and primarily by surface contamination. Although strict sanitary practices were employed to avoid purposeful contamination, this did not appear to reduce the amount of contamination. Factors such as wind-

blown sand (Ridings, unpublished data), animal trackings, and surface water runoff could have served as major means for contamination. Untreated soil from alleyways between the plots also could have been a major source of contamination. The recontamination appeared more severe in test area B which was level than in test area A which was mostly on a slope. The effect of tarp thickness was not evaluated because the differences between test area A and B mentioned above were confounded with the tarp thickness effect. In general, the sooner a plot was invaded by *P. parasitica* the more serious the root rot damage in that plot. This was evidenced by the regression equation ( $RRR = 1.27 + .51 RRT$ ). The control plots showed the least root rot where *P. parasitica* was minimal. After 12 months, *Fusarium* spp. and *Pythium* spp. were recovered from all fumigated plots, and *P. parasitica* and *G. macrocarpus* were detected from at least 18 of the 21 fumigated plots. The late detection (at 12-month sample time) of *P. parasitica* in the soil and roots of seedlings indicated that contamination had occurred, but root rot was not easily seen upon examination of the root system. Thus, these seedlings could be transplanted as liners without any obvious root rot problem and consequently contamination of the liner bed would be immediate. Thus, the use of seedlings with apparently healthy roots may be inadequate for assuming the absence of a pathogen in the roots. Although the soil-borne pathogens *P. parasitica*, *F. oxysporum*, and *F. solani* accounted for 60% of the root rot by regression analysis, *P. parasitica* was the major contributor (56%) of this loss. The remaining 40% of the root rot appeared to be related to possible non-biological factors such as poor soil aeration (10). The role of *P. parasitica* in affecting the root rot rating ( $R^2 = 0.56$ ) was not as great as its effect on the tap root length ( $R^2 = 0.69$ ). *Phytophthora parasitica* is definitely established as a pathogen of citrus roots (3, 10, 14, 17, 18), and the severity of attack by *P. parasitica* may be influenced by the aggressiveness of the isolate (6), source of nitrogen fertilizer (8), susceptibility of rootstock (4, 9), inoculum density, availability of moisture for infection, and competitive nature of other microorganisms in the soil environment (2). The pathogenicity of the isolates of *P. parasitica* from these test plots proved highly virulent to Carrizo seedlings in greenhouse tests (Ridings and Schenck, unpublished data).

The ability of the Carrizo seedlings to develop in the fumigated plots without VA mycorrhizal fungi evidently was due to the adequate available phosphorus (16). *Glomus macrocarpus* was slow to reestablish and therefore did not appear to influence growth appreciably. Although infection by *G. macrocarpus* occurred early in the control plots, there did not appear to be any overall protection against pathogen infection or decrease in root rot development especially where *P. parasitica* was present. However, further work with the VA mycorrhizae established prior to the pathogen would help elucidate any protective mechanism against *P. parasitica*.

Although effective kill by  $CH_3Br$  (as Brozone) appeared adequate in this test, good growth by seedlings was not ob-

tained unless early contamination especially by *P. parasitica* was prevented. It appears that in nurseries with similar cultural practices, the large part of the root rot complex may best be controlled by reducing contamination and/or protecting seedlings growing in fumigated soils. This might be accomplished with pesticides and/or manipulation of conditions which would favor less or no infection such as judicious use of water, the use of nitrogen sources which disfavor infection, employment of more resistant seedlings as rootstocks, and the elimination of sources of contamination. The remaining percentage of the root rot problem might be reduced with better planting sites, more frequent cultivation, and/or better spacing of seedlings.

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