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METHOD FOR RAPID SCREENING OF FUNGICIDES AS CANDIDATES FOR CITRUS MELANOSE CONTROL[1]

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Abstract. **A method is described for screening materials for melanose control without having to subject them to costly and time consuming conventional-type field plot tests, that can provide reliable data only in years when natural infection is sufficiently heavy. Young grapefruit trees were sprayed with test materials about 4 weeks after petal fall and samples of fruit were picked and inoculated in the laboratory with conidia of the melanose fungus,** Diaporthe citri, **to determine the fungicidal activity of the spray residue. The inherent activity of a material against** D. citri **was determined from samples tested soon after treatment. Information on fungicide stability and resistance to weathering was obtained from additional tests on fruit sampled at longer intervals of up to 7 weeks after spraying.**

Several practical problems are encountered in Florida when attempting to determine the potential value of new materials for melanose control in conventional-type plot tests in citrus groves.

Melanose severity varies considerably from year to year and sometimes there is insufficient disease pressure to determine whether a material even has enough activity to justify inclusion in further trials.

Generally, serious losses from melanose are encountered only in older groves. Because of the considerable difference in vigor that can occur in older groves between trees, and the influence this can have on the abundance of inoculum-bearing dead twigs, melanose pressure can be extremely variable. To compensate for this, it is necessary to increase the number of trees per plot and the number of replications per treatment. Another problem that arises in older groves is that, because of tree size, buffer trees are essential between plots to guard against spray drift. Thus, a large area of grove is required to conduct even small scale plot tests for melanose control.

The number of treatments that can be conveniently included in each grove test is determined by the amount of handgun spraying that can be handled in one day. In practice, it is usually possible to increase the number of treatments only at the expense of reduced plot size and replication. Because of the high costs and time involved in testing fungicides against melanose in conventional-type plots, a more rapid and cheaper method was sought for screening candidate materials for melanose control.

This paper describes a field-laboratory bioassay procedure (i) to screen out very rapidly those materials that possess little or no inherent protective activity against infection by *Diaporthe citri* Wolf when applied to fruit rind and (ii) to determine whether a spray residue on fruit rind is likely to persist long enough to provide melanose control when the material is applied only once postbloom.

Materials and Methods

When a young unsprayed citrus fruit, with portion of stem attached and leaves removed, is placed with its stem in water and inoculated with *D. citri,* a melanose reaction develops within 7 days, long before substantial degeneration of the fruit commences.

Spraying of trees. Grapefruit trees ('Marsh' and 'Ruby') were sprayed once to drip off with the test materials between mid-April and early May, approx 4 weeks after petal fall. Only 2 trees were used for each treatment, if they carried sufficient fruit for sampling purposes.

Fungicides. The fungicides used were basic copper sulfate, Cu 53% (Cities Service Co.); captafol (Difolatan 4F, Chevron Chemical Co.); dithianon (Thynon W75, Thompson-Hayward Chemical Co.); chorothalonil (Bravo 6F, Diamond Shamrock Chemical Co.); captan (Orthocide 80W, Chevron Chemical Co.); folpet (Phaltan 50W, Chevron Chemical Co.); dodine (Cyprex 65W, American Cyanamid Co.); benomyl (Benlate 50W, E. I. duPont de Nemours and Co.); guazatine (SN-513 30EC, Nor-Am Agricultural Products, Inc.) and EL-222 1EC (Eli Lilly and Co.). No adjuvants were used in any of the spray mixes except for captan and folpet, to which Chevron spray sticker was added.

Fungus culture and inoculum production. Stock cultures of *D. citri* were maintained on potato dextrose agar. To produce the conidia that are required as inoculum, the fungus was grown in culture tubes on portions of autoclaved 1- to 2-year-old living twigs cut from grapefruit trees. Portions of stock culture were transferred to the stems and these started to produce mature pycnidia 2 to 3 weeks later. After pycnidia development was adequate, the colonized

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stems were air-dried for 2 days in the laboratory and then placed on damp filter paper in a petri dish to induce exudation of conidia tendrils.

After 3 days the tendrils were picked off with a needle and deposited in a 1% solution of filtered orange juice in distilled water. A small amount of orange juice was included in the medium because conidia germination on the rind surface was sometimes very poor if only distilled water was used. A 1% orange juice solution assured good infection while a concn of orange juice in excess of 5% reduced infection. The conidia dispersed rapidly from the tendrils but soon sank and adhered to the bottom of the container. Frequent dislodgement with a small camel's-hair brush was needed to maintain a uniform suspension for inoculation purposes. The concn of the inoculum was adjusted to 5×10^5 conidia/ml prior to use.

Preparation of detached fruit samples for inoculation. Fruit that could be reached from the ground were clipped, with 4 to 6 cm of the stem attached, at random from each tree. At all times during this and subsequent operations, the fruit were held only by their stems to prevent removal or smudging of the spray residue.

As soon as each fruit was picked, it was placed on a 3-cm high hardware cloth (mesh size 12 mm) platform in a plastic wash basin that measured 32 cm x 26 cm x 14 cm deep (Fig. 1). The stem of each fruit was inserted through the mesh and the fruit were placed close enough together to prevent possible subsequent change in their orientation. As a further precaution against fruit movement after inoculation, the platforms were always filled to capacity. The total number of fruit sampled from each tree always exceeded 18.

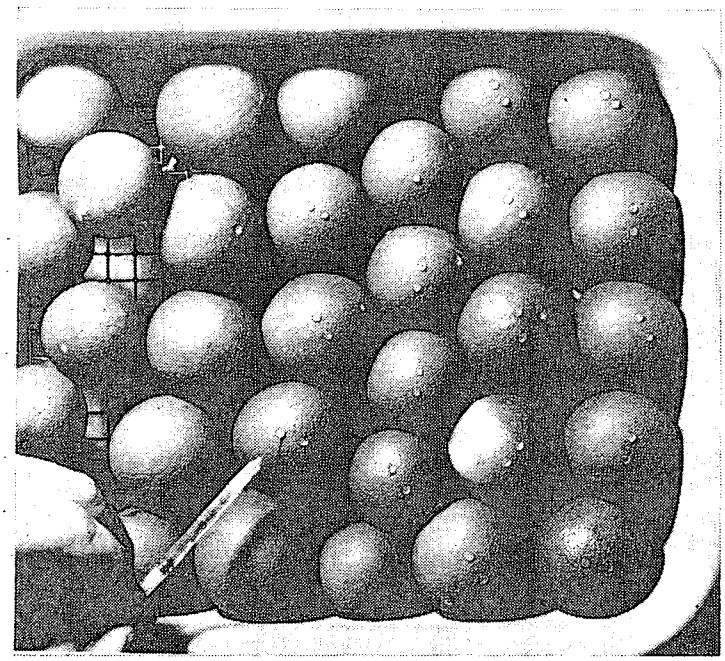

Fig. 1. Sample of fruit clipped from a fungicide-sprayed tree and placed on a hardware cloth platform in a plastic basin, with stems inserted through mesh into water. Drops of inoculum of *Diaporthe citri* are applied from a syringe to 2 locations near the uppermost point on each fruit, prior to covering the basin with a sheet of polyethylene.

Within 2 hr after picking, the basins were returned to the laboratory and filled with demineralized water to the level of the platform.

Inoculation procedure. On the day of sampling, 2 small drops (each approx 15 μliter) of inoculum were deposited from a syringe at locations about 10 mm apart, near the uppermost point of each fruit (Fig. 1). Care had to be taken to prevent excessive evaporation of the drops, because water is required for conidia survival and germination. The humidity was increased rapidly by directing a fine spray of water momentarily into the basin (without causing run-off) and the basin was covered immediately with a polyethylene sheet. The temp of the laboratory ranged from 25 to 28C. Two days after inoculation, the covers were removed and, in the absence of a fungitoxic residue, disease symptoms developed within the next 4 to 7 days.

The conidia concn was high enough to produce a continuous necrotic spot over the areas contacted by the drops of inoculum (Fig. 2). In assessing the results, a fruit was regarded as lacking an effective fungitoxic residue even if symptoms developed at only one of the two inoculation sites.

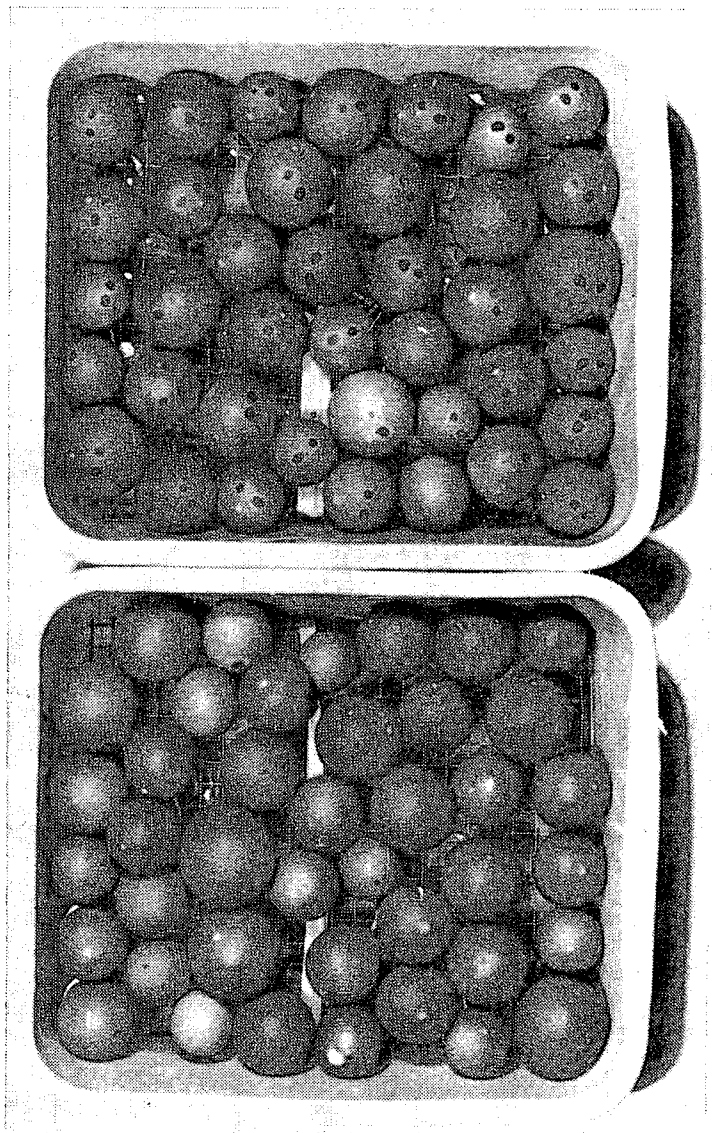

Fig. 2. Appearance of necrotic spots on rind at locations where drops of inoculum of *Diaporthe citri* were applied 7 days previously. Top basin contains fruit from unsprayed tree. Bottom basin contains fruit from tree sprayed 3 weeks previously with basic copper sulfate.

Results and Discussion

Relatively high inherent fungitoxicity to *D. citri* was exhibited by basic copper sulfate, Difolatan, dithianon, Bravo, captan and Phaltan but not by the other materials tested (Table 1). At 4 to 7 weeks after application, Difolatan and dithianon still provided good protection, either equal to or superior to that provided by basic copper sulfate. Bravo

Table 1. Relative protectant capability of fungicide residues on grapefruit rind against melanose attack with time after postbloom spray application.

Material and rate per 100 gal[z]		Detached fruit remaining melanose-free following inoculation with *Diaporthe citri* at various intervals after spraying (%)											
		1975				1976				1977			
		Test 1		Test 2		Test 1		Test 2		Test 1		Test 2	
		18[y]	35	14	28	15	37	7	35	19	50	33	42
Basic copper sulfate	1.5 lb.	100a[x]	83a	100a	98a	92a	73b	100a	67b	86b	52b	80a	50b
Difolatan 4F	1.0 quart	—	—	—	—	100a	100a	100a	100a	100a	98a	100a	100a
Dithianon 75W	1.0 lb.	100a	93a	97a	90a	100a	92a	—	—	100a	84a	100a	91a
Bravo 6F	0.17 gal	100a	17b	90a	26b	100a	41c	—	—	100a	31b	97a	54b
Captan 80W	1.25 lb.	—	—	—	—	96a	33cd	100a	29c	—	—	—	—
Phaltan 50W	0.4 lb.	—	—	—	—	67b	7e	100a	11c	—	—	—	—
Cyprex 65W	2.0 lb.	—	—	—	—	—	—	—	—	59b	—	36b	29c
Benlate 50W	0.5 lb.	41b	6b	20b	27b	37c	20de	—	—	—	—	—	—
SN-513 30EC	8.0 oz	—	—	31b	—	—	—	57b	—	—	—	—	—
EL-222 1EC	11.2 oz	—	—	—	—	—	—	—	—	23c	—	0c	—
Check (unsprayed)		12c	6b	14b	28b	11d	10e	2c	16c	0d	21b	3c	14c
Accumulated rainfall between spraying and sampling (inches)		0.15	6.97	0.30	1.30	3.18	10.98	0.27	8.73	0.52	5.25	2.67	4.10
Date of spraying		April 14		April 16		April 27		April 28		April 28		May 4	

[z]Amount of formulated product.
[y]Number days between spray application and bioassay.
[x]Values within a column followed by the same letter are not significantly different at the 5% level.

usually exhibited a shorter period of residual activity than basic copper sulfate. Captan still provided good protectant action at 2 weeks after spraying but not at 5 weeks. Results with Phaltan were even poorer than with captan.

The data obtained from the field-laboratory bioassay method for determining the loss of activity of spray residues against *D. citri* with time after spraying have correlated well with the relative levels of melanose control obtained in conventional field tests. In Florida, postbloom sprays of basic copper sulfate and certain other copper fungicides have generally given better control of melanose than similarly timed sprays of Benlate or Bravo (6). Dithianon performed as well as copper fungicides in those few field tests in which it was included (6, 7). In Texas, Difolatan, applied postbloom, was equal to, or better than copper fungicides for melanose control (4, 5).

Benlate performed better in some Florida (2, 6) and Texas (4) field tests than would have been expected from the results of the bioassay tests. This could be explained on the basis that although Benlate sprays have little protectant action against melanose, they can reduce the inoculum supply from dead twigs (8). However, in Florida this eradicant effect has not been of sufficient magnitude to justify use of Benlate as a practical alternative to the currently used copper spray.

Bravo has given promising results in some tests (2) but poor results in others (4, 6), and generally it has been less effective than copper. The data presented here and elsewhere (8) indicate that Bravo provides relatively short residual activity. However, the rate of loss of activity did not correlate closely with accumulated rainfall totals. For example, Bravo had completely lost its protectant effect after 4 weeks and 1.30 inches rain in Test 2 of 1975 (Table 1). Yet, in Test 2 of 1977, after 6 weeks and 4.10 inches rain, it still possessed a substantial protective capability.

The very poor residual activity exhibited by Cyprex and Phaltan (Table 1) is consistent with the poor field performance of these materials previously reported (1).

For economy, only one spray is normally applied in Florida for melanose control (3). Because climatic conditions favorable for fruit infection seldom occur in March or April, a single spray application is best delayed until late April or early May (6, 9). Except in years when the bloom is unusually early, this means that the single spray should be applied 4 to 6 weeks after peak bloom. In Florida the rind does not become immune to melanose attack until 10 to 12 weeks after petal fall (9). Thus, even when spraying is delayed until 4 to 6 weeks after bloom, a long protective action is still essential to assure satisfactory melanose control. Few fungicides possess such a capability, which is one of the reasons why it has been impossible to replace postbloom copper sprays for the control of this disease. The only other materials that consistently provided long-term protection against melanose when applied postbloom have been Difolatan and dithianon. Unfortunately, Difolatan sometimes injures fruit rind and it can also injure young shoots. Therefore, Difolatan can only be safely applied after mature fruit have been picked and before spring shoot growth commences. To obtain satisfactory control of melanose with a late dormant Difolatan spray, massive amounts of material have to be applied (6, 7). The high costs of such treatment normally renders this method of control impractical. Dithianon could be a potential alternative to copper fungicides for melanose control but this material is not registered for use on citrus or any other crop in the U.S.A.

Feasibly, other materials such as Bravo or captan might provide acceptable control if applied more than once during the fruit susceptible period, but the costs could be prohibitive.

The bioassay test described in this paper provides a rapid method for determining whether a candidate material has sufficient inherent fungitoxicity against *D. citri* to justify inclusion in further tests. It also provides a convenient means for measuring the fungitoxic persistence of deposits under field conditions, as affected by rates of chemical degradation or volatilization and physical erosion by rain or wind. This method can therefore reveal, in one season, whether a candidate material has sufficiently long protective action under natural conditions to justify further testing and development for melanose control.

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REINVASION OF METHYL BROMIDE TREATED SOIL BY SOIL-BORNE FUNGI AND THEIR SUBSEQUENT EFFECT ON CITRUS SEEDLING GROWTH[1,2]

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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 m²). 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) 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 CH_3Br 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 CH_3Br (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 CH_3Br (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% CH_3Br, 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) CH_3Br (as Brozone) per 100 sq. ft. (9.29 m²). Fumigation of plots in test area B was done by releasing

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