Detection of Captan Residues in Prune Fruits and Blossoms by Thin-Layer Chromatography

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Captan [N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide], produced by the Chevron Chemical Company, Ortho Division, is a general fungicide for treatment of fruit, foliar, soil, and seed-borne diseases. Methods for detecting captan include colorimetry (5, 3) and gas chromatography (4, 1) utilizing the electron capture detector. The colorimetric methods can detect 5 to 10 µg, while the gas chromatographic method can detect 0.5 nanogram.

The thin-layer chromatographic (TLC) method described below was developed as a rapid screening procedure for the detection of captan residues in the presence of difolatan residues [N-(1,1,2,2-tetrachloroethylsulfenyl)-cis Δ^4-cyclohexene-1,2-dicarboximide]. The method can selectively detect 1 µg of captan since difolatan does not react with the resorcinol spray reagents.

Experimental

Plant material samples. The fruit sources, harvest procedures, dipping and dehydration procedures, and storage history of the prune fruits have been described (1). Fruits were dipped in a captan mixture (Orthocide 50W) at 15 to 21° C so that each dip concentration was 1, 2, or 4 lbs. of active ingredient per 100 gallons of solution. The captan-water mixture was agitated by hand for three minutes, and duplicate samples of fruit were dipped in each concentration. A second series of fruit was dipped in a similar manner for dehydration. Fruits were dipped in water as controls.

Dipped fruit was air-dried for 15 minutes at temperatures of 21 to 31° C. One-half of the air-dried fruit was stored

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at 2 ± 0.1° C until analyzed; the other half was dehydrated at 88 to 91° C for 16 hours in a parallel-flow tunnel in the normal commercial manner, and stored at 2 ± 0.1° C until analyzed.

Prune blossoms on orchard trees were sprayed at three intervals with conventional high-volume speed-spray equipment. Orthocide 50W in water was applied at the rate of 4 lbs. captan actual in 350 gallons water per acre. The first application was on March 9 (green bud stage), the second on March 18 (popcorn stage), and the third on March 26 (full bloom near petal fall stage). Usually blossoms were sampled immediately after spraying; the blossoms were also sampled before spraying except with the first application. To determine captan levels after a period of environmental weathering degradation, a final sampling was taken on April 2 (petal fall stage), without a spray application.

Methods of extraction and cleanup. Either the chopped, pitted, and weighed fruit, or the unchopped blossoms were placed in a 1-liter round-bottom boiling flask, and redistilled benzene was added in the ratio of either 200 g fruit/250 ml benzene for the green fruit, or 100 g fruit/200 ml benzene for the dried fruit, or 150 weighed sets/250 ml benzene for the buds and blossoms. The mixture was shaken for 2 minutes, refluxed for 15 minutes under a Liebig water-cooled condenser (2), cooled in an ice bath, and decanted through Whatman No. 1 filter paper into a receiver flask. Fresh benzene was added to the plant material, and the above process was repeated for a total of 3 extractions. The pooled benzene extracts were concentrated in vacuo at 50 to 60° C.

Cleanup of the plant materials was accomplished in the following manner. A glass column of 25 x 250 mm with a 300-ml solvent reservoir at the top was packed with a small glass-wool plug at the bottom, 1" of anhydrous sodium sulfate powder lightly tamped, 4" of florisil (activated at 270° C for 3 hours) lightly tamped, and 1" of anhydrous sodium sulfate powder. The column was wetted with 100 ml of redistilled benzene, and all the benzene was discarded except 20 ml on the column top.

The concentrated extract (5 grams in 5 to 10 ml) was transferred to the column and allowed to flow into the column packing followed by two 25 ml rinses of benzene and 130 ml of a premixed solution of 2% redistilled isopropyl alcohol and 98% redistilled benzene. After the first volume of eluting solution had passed into the column, 230 ml additional eluate was collected. The eluates were pooled in a 1-liter round-bottom boiling flask and were concentrated in vacuo at 50 to 60° C. The samples were transferred to a 6.5 ml sedimentation tube (McNaught and McKay-Shevky-Stafford or equivalent) with benzene, evaporated to 125 µl with the aid of warm air, and mixed. The extractives from 4 grams or less of plant material were spotted on the TLC plates. Captan fortified at a level of 1 ppm in untreated plant material resulted in recoveries ranging from 86 to 89% when checked by gas chromatography (GLC) (1).

Methods of TLC Plate Preparation. Forty grams of Silica gel H (Brinkmann Instruments Inc.) was mixed in 92 ml of distilled water by stirring. This amount of slurry will usually coat five 8 x 8" glass plates with a 500-micron-thick layer of silica gel. The spread layers were allowed to air-dry for 1 hour and then heated for 30 minutes at 100° C. The activated plates were stored in a desiccator.

Spotting. Four grams or less of plant extractives containing 1 µg or more of captan were applied to the silica gel. The plate was marked 15 cm from the origin for maximum solvent travel. Captan standards in benzene (1 µg/µl) were spotted as references.

Solvent Development. Three hundred ml of 4% isopropyl alcohol-96% benzene were added to the 9 x 3 1/2 x 9" stainless-steel chromatography tank (Thomas-Mitchell TLC tank). The inner walls of the tank were lined with 8 x 8" Whatman No. 1 filter paper to equilibrate the tank atmosphere with solvent vapor. Plates containing the samples were placed in the TLC tank, and the solvent was allowed to ascend to the 15-cm mark at the plate top. The time required for the solvent travel was usually 45 minutes. Plates were air-dried 10 minutes in a hood.

Chromogenic Reagents and Color Development. Two spray reagents were used in detecting the captan. The preferred reagent was resorcinol (7.25 g) in glacial acetic acid (35 ml), but tetraethyl ammonium hydroxide (3 ml of 40%; K&K Labs., Inc., Hollywood, Calif.) added to pyridine (3 ml water plus 24 ml pyridine; J. T. Baker Chem. Co. or equivalent) can also be used when well mixed. Both sprays produced an intense yellow captan spot on a white background. The color for the tetraethyl ammonium hydroxide-pyridine spray reagent developed almost immediately at room temperature, but soon faded. The color for the resorcinol-glacial acetic acid spray developed only after the plate was sprayed and heated. Approximately 1 µg of captan was detected with either spray reagent, and the R_f value was 0.44 in the described developing solvent.

Results and Discussion

Experimental data demonstrate that results with the prune fruit were best when not more than 5 grams of extractives were cleaned up on the florisil and not more than 4 grams of the cleaned up extractives were spotted on the TLC plates. If more material was spotted, foreign extractives caused some streaking or retarding of the captan.

The pyridine-tetraethylammonium hydroxide spray reagent developed an intense yellow color with captan at room temperature, though the color faded within approximately 30 minutes. The reagent did not appear to react with botran (2,6-dichloro-4-nitroaniline), but since botran itself is yellow, high concentrations gave a yellow spot. Botran (R_f 0.53) and captan (R_f 0.44) will separate in the isopropyl alcohol-benzene solvent system. Difolatan with this spray reagent has an orange-colored spot; but with an R_f value of 0.47, captan and difolatan cannot be separated in the solvent system employed. The resorcinol-glacial acetic acid spray reagent (16.7% resorcinol) produced no immediate visible spot at room temperature when the TLC plate was sprayed with it, but the color developed to a stable spot of very intense

yellow when the plate was heated for 20 minutes at 100° C. The minimum heating time was approximately 20 minutes, and the spot did not intensify on further heating. Plates could be preserved for several days in the dark; otherwise the plate background darkened slowly. Difolatan did not react with this spray reagent to produce color. Botran did not appear to react with this reagent. The only interference from botran was the color of botran itself; and, if the concentration was low (<7 μg), little if any color from botran was visible. Since the isopropyl alcohol benzene solvent system separated captan and botran on the TLC plate, the botran color problem was minimized.

Table I shows residue data obtained by GLC for green and dehydrated prune fruit after extraction and cleanup procedures described above. These samples were analyzed by TLC and figure 1 is a photograph of the TLC plate obtained. The spots are explained by the consecutive number code in Table II.

TABLE I

Captan Levels on Postharvest Dipped Prune Fruit Before and After Dehydration[1]

Postharvest Dip: Captan	Green Fruit		Dehydrated Fruit	
Active Ingredient (lb.)/100 gallons	Per Cent Moisture	Ppm[2] Captan	Per Cent Moisture	Ppm[2] Captan
1	73	3.6	30	2.3
2	73	4.8	30	6.1
4	73	11.0	30	11.1

[1]Dehydration was for 16 hours in a parallel-flow tunnel, operated at 88-91° C, dehydration ratio 2.3:1.

[2]Ppm fresh weight, excluding pit weight, not corrected to ppm dry weight, and determined by GLC analysis.

TABLE II

Spot Identification and Semi-Quantitative Ppm Captan Estimated on Prune Fruit on TLC Plate in Figure 1

Spot No.	Sample Code	Per Cent Moisture	Captan Applied[1]	µg Captan Spotted	Ppm[2] Estimated
19	Reference Standard	-	-	5	-
20	Green Fruit	73	1 lb.	12	4
21	Green Fruit	73	2 lb.	8	5
22	Green Fruit	73	4 lb.	22	11
23	Reference Standard	-	-	10	-
24	Dehydrated Fruit	30	1 lb.	6	3
25	Dehydrated Fruit	30	2 lb.	12	6
26	Dehydrated Fruit	30	4 lb.	26	11
27	Reference Standard	-	-	15	-

[1]Applied as a postharvest dip before dehydration with captan rate as lb. actual per 100 gallons water.

[2]Ppm fresh weight, not corrected to ppm dry weight, and estimated from TLC plates with reference to GLC data.

Table III shows residue data for prune buds and blossoms obtained by the same GLC method. These samples were analyzed by TLC and Figure 2 is a photograph of the TLC plate obtained. The spots are explained by the consecutive number codes in Table IV.

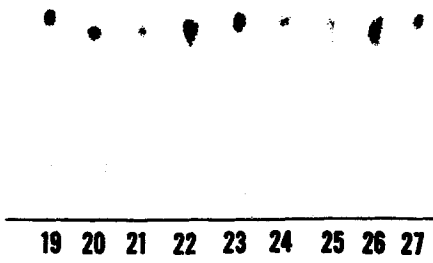

Fig. 1. Green and dehydrated prune fruit extracts chromatographed on Silica gel H in 4% isopropyl alcohol-96% benzene and sprayed with resorcinol-glacial acetic acid reagent. See Table II for explanation.

TABLE III

Captan Levels on Prune Blossoms After Multiple Spray Applications

High Pressure Ground Spray	Prune Blossoms from Green Bud to Petal Fall Growth Stages		
Date Captan Application[1]	Sampling Date	Ppm[2] Captan	Per Cent Moisture
3/9/68	3/13/68	305	78
--	3/18/68	32	82
3/18/68	3/18/68	502	82
--	3/26/68	185	81
3/26/68	3/26/68	652	81
--	4/2/68	448	77

[1]Captan 4 lb. actual per 350 gallons water per acre as Orthocide 50W; same orchard sprayed at each date of application.

[2]Ppm fresh weight not corrected to ppm dry weight, and determined by GLC analysis.

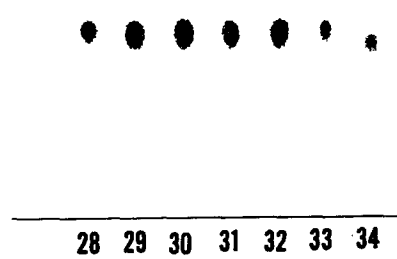

Fig. 2. Prune buds and blossom extracts chromatographed on Silica gel H in 4% isopropyl alcohol-96% benzene and sprayed with resorcinol-glacial acetic acid reagent. See Table IV for explanation.

TABLE IV

Spot Identification and Semi-Quantitative Ppm Captan Estimated on Prune Buds and Blossoms on TLC Plate in Figure 2

Spot No.	Sample Code	Per Cent Moisture	Captan Applied	µg Captan Spotted	Ppm[2] Estimated
28	Reference Standard	-	-	25	-
29	Green Bud	78	4 lb.	56	337
30	Popcorn	82	4 lb.	79	523
31	Reference Standard	-	-	50	-
32	Full Bloom	81	4 lb.	97	648
33	Petal Fall	77	-	77	516
34	Reference Standard	-	-	75	-

[1]Applied as a high-volume ground spray with captan rate as actual lb. per 350 gallons water per acre.

[2]Ppm fresh weight not corrected to ppm dry weight, and estimated from TLC plates with reference to GLC data.

The captan spots in Figures 1 and 2 were well developed, resolved from the origin and foreign materials in the plant extractives, and have consistent R_f values within reasonable limits of experimental error. Thus the TLC technique described provides a rapid, reliable screening procedure for detecting captan in the presence of difolatan. With precautions as outlined above, screening for botran could also be included in the presence of captan and difolatan.

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