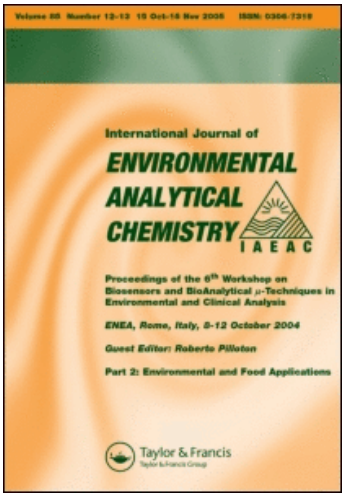

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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C. Sanchez-brunete[a]; A. De Cal[a]; P. Melgarejo[a]; J. L. Tadeo[a]
[a] Department of Plant Protection, C.I.T.-I.N.I.A., Crta. de la Coruña, Madrid, Spain

To cite this Article Sanchez-brunete, C. , De Cal, A. , Melgarejo, P. and Tadeo, J. L.(1989) 'Determination of Fungicide Residues in Peach Trees', International Journal of Environmental Analytical Chemistry, 37: 1, 35 — 43
To link to this Article: DOI: 10.1080/03067318908026883
URL: http://dx.doi.org/10.1080/03067318908026883

Intern. J. Environ. Anal. Chem., Vol. 37, pp. 35–43
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DETERMINATION OF FUNGICIDE RESIDUES IN PEACH TREES*

C. SANCHEZ-BRUNETE, A. DE CAL, P. MELGAREJO and J. L. TADEO

Department of Plant Protection, C.I.T.–I.N.I.A., Crta. de la Coruña, 28040 Madrid, Spain

The residue analyses of three fungicides, thiram, captan and benomyl, have been accomplished in peach trees. Thiram was extracted with hexane:ethyl acetate (80:20 v/v) and determined after a column clean-up by reverse-phase HPLC with a mixture of acetonitrile:0.3 ammonia at 280 nm. Captan was extracted with acetonitrile:water (2:1) and determined by GLC–ECD after a partition and a column clean-up. Benomyl was extracted with ethyl acetate and determined, as MBC, by reverse-phase HPLC with methanol:0.3 ammonia, after a partition and a column clean-up.

Recoveries through the methods were always higher than 70%. The detection limits were 0.5 ppm for thiram, 0.02 ppm for captan and 0.2 ppm for benomyl, based on a 5 g sample.

Residue levels in peach twigs, treated with fungicide solutions, are reported.

KEY WORDS: Thiram, captan, benomyl, residues, peach trees.

INTRODUCTION

Previous studies on the epiphytic mycoflora of peach twigs and flowers showed that it includes beneficial fungi that, by antagonistic interactions with peach pathogens, could inhibit infection and pathogenesis.[1] The impact of pesticides on these non-target microorganisms constitutes one type of side effect that is of fundamental ecological significance due to the changes produced in the ecosystem and their implications in the development of diseases.[2,3]

Several fungi (*Monilinia* spp, *Taphrina* spp, *Clasterosporium* spp etc.) induce important diseases in peach trees and different fungicides, several times during the year, are used to control fungal pathogens in our main producing areas. Therefore, the study of residues left by these chemicals on the tree and their effect on microorganisms is basic to achieve a suitable pesticide schedule and to approach to integrated control strategies.

Three of the more used fungicides have been selected in this study, thiram (tetramethyl thiuram disulphide), captan (N-(trichloromethylthio)-cyclohex-4-ene-

*Presented at the 18th International Symposium on Environmental and Analytical Chemistry, Barcelona, 5–8 September 1988.

1,2-dicarboximide) and benomyl (methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate).

The aim of this work was to study the analytical determination of the residues of these fungicides in twigs of peach trees along the season.

EXPERIMENTAL

Apparatus and Equipment

The liquid chromatograph used was a Beckman model 421 A equipped with a 20 μl loop injector, a 4.6 × 250 mm stainless steel column (RP18 reverse-phase, 5 μm), a 160 A fixed wavelength UV detector, and a Spectra-Physics Sp 4290 integrator. A 55:45 mixture of acetonitrile and 0.3% ammonia was used as eluent for thiram and a 60:40 mixture of methanol and 0.3% ammonia for benomyl; the solvent flow rate was 1 ml/min, the temperature ambient, the injection volume 20 μl and the detection wavelength 280 nm. The gas chromatograph used to determine captan was a Varian Aerograph 3700 equipped with an ECD ^{63}Ni and a glass column packed with 3% OV-17 on Chromosorb W-HP (80–100 mesh). The detector and injector temperature were 300 °C and 230 °C respectively. The column-oven temperature was 190 °C. The carrier gas was nitrogen at a flow rate of 40 ml/min. Homogenization was made in a Du Pont Sorvall Omni-mixer.

Reagents and Samples

The reagents employed in this study were sodium sulfate (anhydr.), sodium chloride and ammonia, all AR grade (Merck). Solvents used were acetonitrile, diethyl ether, ethanol, ethyl acetate, hexane and methanol all AR grade (Merck). Column adsorbents used were silica gel 60, 0.063–0.200 mm, (Merck) and florisil, 60–100 mesh, (Serva).

The analytical thiram standard (99% purity) was obtained from Riedel-de Haen (Hannover, FRG), captan (99% purity) from Shell London, UK) and benomyl (99% purity) from E. I. Du Pont (Wilmington, Delaware, USA).

The trees used were 5-year old peach trees cv. Baby Gold, located in Montañana (Zaragoza, Spain). Two experimental plots, each containing eight trees, were established in the orchard. One of the plots received the fungicide treatments while the other one was unsprayed. Fungicides were applied in aqueous solutions, containing 1600 ppm thiram (1.44 Kg ha^{-1}), 1250 ppm captan (1.13 Kg ha^{-1}) and 250 ppm benomyl (0.23 Kg ha^{-1}), with a Matabi sprayer to run-off. Ten twigs of about 20 cm long, three replicates per treatment, were sampled at random one week after treatment and kept frozen at −18 °C until analysis.

Procedure

Sample preparation and extraction Twigs were divided into pieces. A represen-

tative 5 g sample was weighed into the 100 ml flask of a Sorvall homogenizer. Thiram was extracted with 20 ml of ethyl acetate:hexane (20:80 v/v), captan with 20 ml of acetonitrile:water (2:1) and benomyl with 20 ml of ethyl acetate. The mixture was homogenized for 2 min at high speed and filtered under vacuum through Whatman No. 1 filter paper, using a Büchner funnel. The extraction was repeated twice, the extract was transferred to a 100-ml round-bottomed flask and the organic solvent was removed under vacuum on a rotary evaporator.

Clean-up Thiram: The residue left after the solvent evaporation was dissolved in hexane and interfering coextractive substances eliminated through a silica gel column (5 g), the first fraction eluted with 20 ml of 15% ethyl ether:hexane discarded and thiram eluted with 50 ml of 50% ethyl ether:hexane. The organic solvent was removed under vacuum, the residue dissolved in acetonitrile and transferred to a 10 ml tube, which was stored until ready for HPLC analysis.

Captan: The residue was dissolved in methylene chloride and partitioned three times between methylene chloride (50 ml) and 2% NaCl (40 ml) for 2 min. The aqueous phase was discarded and the methylene chloride was evaporated after passing through anhydrous Na_2SO_4. The residue was dissolved in hexane and chromatographed through activated florisil, according to the procedure of Mills *et al.*[6] Captan was eluted with 200 ml of 50% methylene chloride, 1.5% acetonitrile, 48.5% hexane. The organic solvent was removed under vacuum, the residue dissolved in hexane and transferred to a 10-ml tube which was stored until ready for GLC–ECD determination.

Benomyl: The residue was dissolved in 10 ml of 0.1 N HCl, the solution transferred to a 100-ml separating funnel, washed twice by shaking with 20 ml hexane for 2 min and the hexane discarded. The aqueous layer was basified with 5 ml of 1 N NaOH and extracted with three 20 ml portions of ethyl acetate, shaking for 2 min each time. The aqueous phase was discarded and the ethyl acetate removed under vacuum after passing through anhydrous Na_2SO_4. If a further clean-up was necessary, the residue was dissolved in ethyl acetate and chromatographed through florisil (5 g) with ethyl acetate (15 ml). The organic solvent was removed, the residue dissolved in methanol and transferred to a 10 ml tube which was stored until ready for HPLC analysis.

Detection and determination: The determination of thiram and benomyl has been carried out by HPLC with an UV detector at 280 nm and captan was determined by GLC with an ECD. Fungicide concentrations were calculated by comparing integration counts or peak heights of samples with those of standards.

RESULTS AND DISCUSSION

Thiram

The fungicide was extracted from peach twigs with hexane-ethyl acetate (80:20 v/v) and determined by reverse-phase HPLC with a 55:45 mixture of acetonitrile and

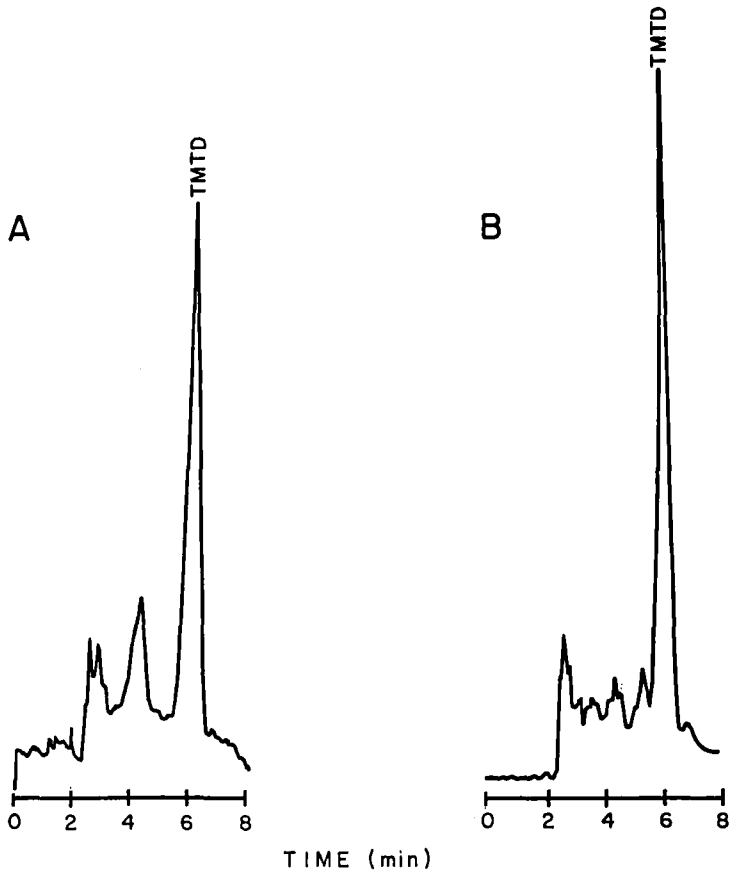

Figure 1 HPLC chromatograms of thiram: (A) Twigs of peach trees treated with 1600 ppm of thiram (60 μg/g). (B) Twigs of untreated peach trees spiked with 10 ppm of thiram (TMTD).

0.3% ammonia, in a way similar to that used to determine thiram residues on lettuce.[4] Nevertheless a column clean-up was necessary at low levels to remove interfering substances and improve sensitivity. This was achieved by using a silica gel column and eluting with ethyl ether-hexane (1:1 v/v). In Figure 1, representative chromatograms of treated and spiked samples are shown. The response was linear from 0 ppm to at least 20 ppm. The detection limit was 0.5 ppm, based on a 5 g sample. Recoveries through the method were always higher than 70% (Table 1).

Fungicide levels in old twigs, one week after two monthly treatments of 1.6 g ai/l, were 70 ± 15 μg thiram/g fresh weight (Table 2). Nevertheless, thiram levels in young twigs, grown after the last fungicide application, were undetectable.

Captan

Captan residues in twigs of peach trees were analyzed by GLC–ECD following a

Table 1 Recoveries (%) of added thiram, captan and benomyl from peach twigs

	Added μg/g	*No. of anal.*	*Recovery ± SD*
Thiram	10	4	97 ± 6
	5	4	83 ± 7
Captan	2	4	86 ± 5
	1	4	90 ± 8
	0.5	3	83 ± 8
Benomyl	10	4	98 ± 7
	5	4	97 ± 7
	1	4	96 ± 8

Table 2 Thiram, captam and benomyl residues (ppm) found in peach twigs[a]

Treatment date fungicide	*Sample*	*Residue*	
April			
Thiram	Old twigs	Thiram	70 ± 15
May			
Captan	Young twigs	Captan	3.5 ± 0.7
1st June			
Captan	Young twigs	Captan	20.6 ± 5.1
End June			
Captan	Young twigs	Captan	13.8 ± 2.5
Benomyl	Young twigs	MBC	10.6 ± 1.4
July			
Benomyl	Young twigs	Captan	0.7 ± 0.2
		MBC	10.8 ± 4.2

[a]Samplings were done a week after treatment. Values are the mean of three replicates ± standard deviation. Fungicides were applied in aqueous solutions, containing 1600 ppm thiram (1.44 Kg ha^{-1}), 1250 captan (1.13 Kg ha^{-1}) and 250 ppm benomyl (0.23 kg ha^{-1}), with a Matabi sprayer to run-off. Benomyl has been determined as carbendazim (MBC).

method previously used in apples, grapes and pears.[5] The fungicide was extracted with acetonitrile:water (2:1) and two clean-up procedures were necessary, partition between methylene chloride and 2% NaCl and chromatography through a florisil column, using methylene chloride, acetonitrile and hexane eluting mixtures.[6] In Figure 2, representative chromatograms of treated and spiked samples are shown. The response was linear, at least, in the range 0–4 ppm. Recoveries by the method were always higher than 75% (Table 1). The detection limit was 0.02 ppm, based on a 5 g sample.

Captan levels in young twigs, after the first treatment with 1.25 g captan/l, were 3.5 ± 0.7 ppm and after the two other treatments the levels varied from 11 to

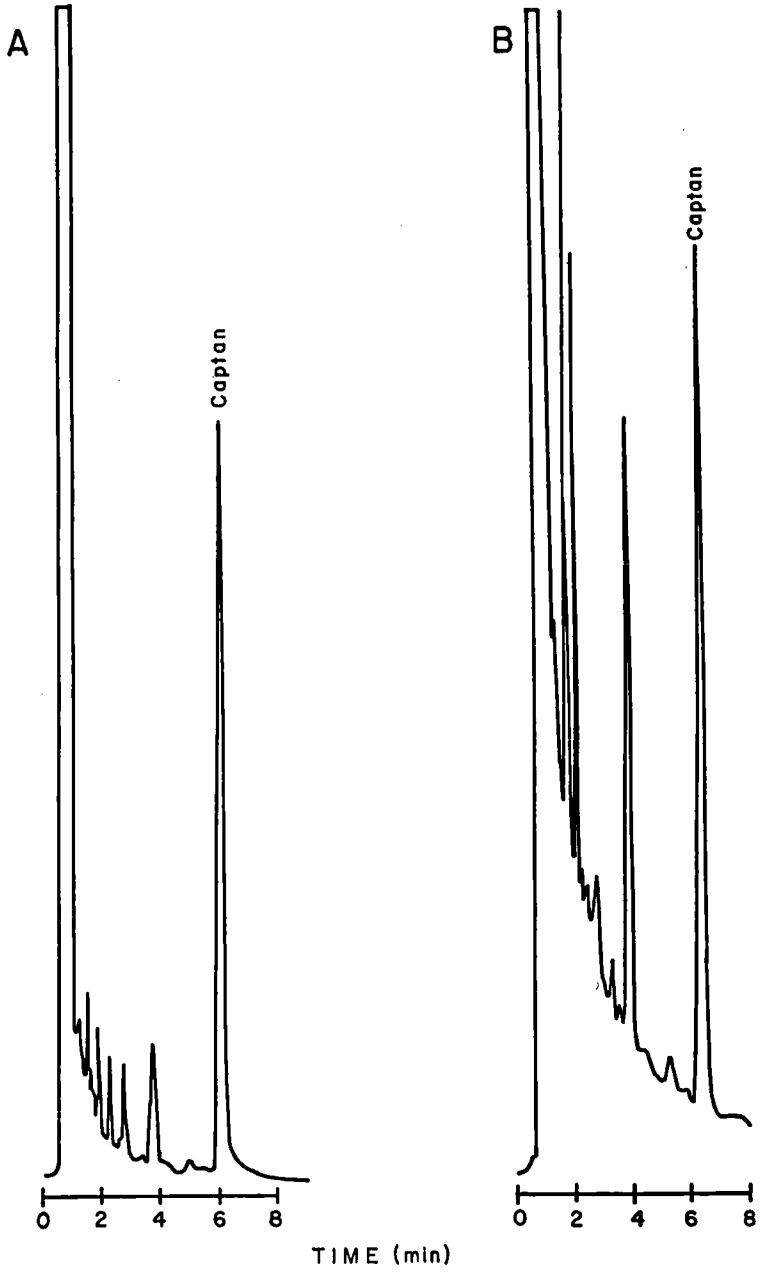

Figure 2 GLC chromatograms of captan: (A) Twigs of peach trees treated with 1250 ppm of captan (3.2 μg/g). (B) Twigs of untreated peach trees spiked with 1 ppm of captan.

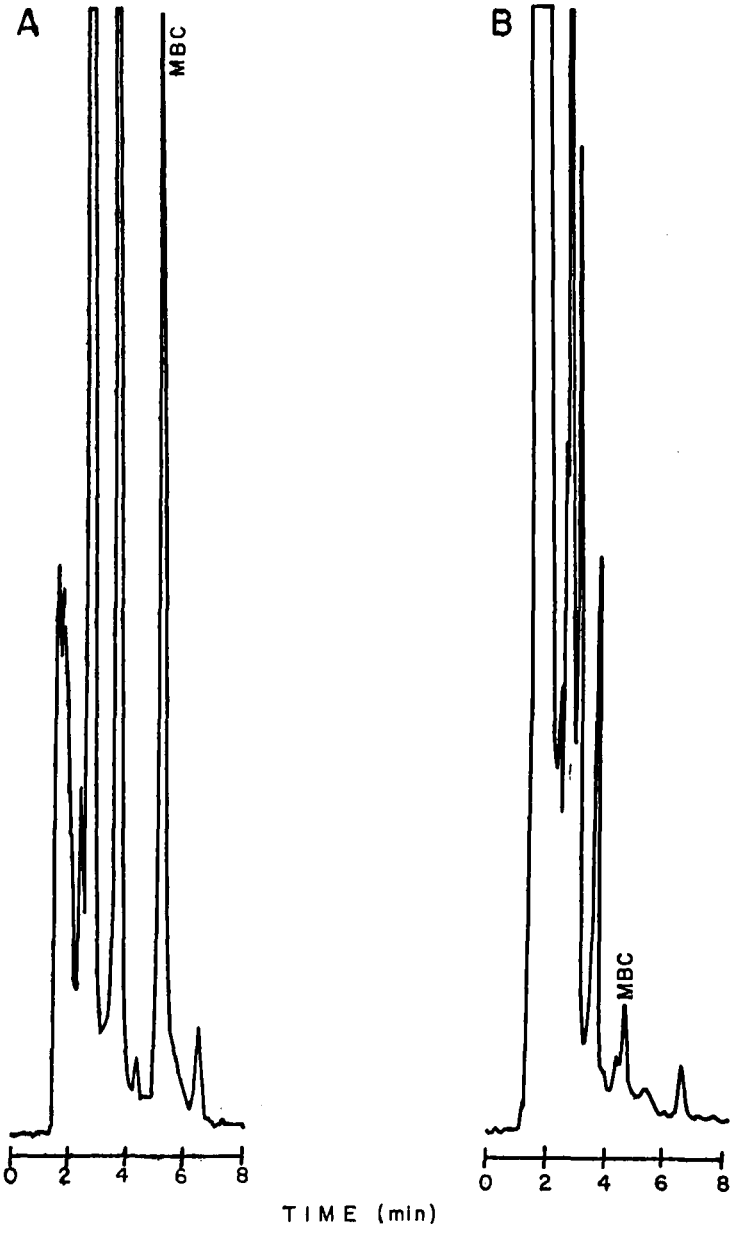

Figure 3 HPLC chromatograms of MBC: (A) Twigs of peach trees treated with 250 ppm of benomyl (10 μg/g). (B) Twigs of untreated peach trees spiked with 0.5 ppm of benomyl.

25 ppm (Table 2). A month after the last treatment, levels decreased to around 1 ppm and two months later were undetectable.

Benomyl

Benomyl is easily transformed to carbendazim under natural and artificial conditions.[7,8] In the ethyl acetate extracts from samples it is fastly decomposed,[9] if not already decomposed at field conditions. The weakly basic property of MBC is used in the partition clean-up, as previously used in the analysis of other bencimidazole and imidazole fungicides.[8,10,11] A further clean-up was accomplished through florisil, eluting MBC with ethyl acetate. MBC was determined by reverse-phase HPLC with methanol and aqueous ammonia (0.3%), in a similar way to imidazoles determination.[11] In Figure 3, representative chromatograms of treated and spiked samples are shown. The response was linear between 0–20 ppm. Recoveries through the method were always higher than 85% (Table 1). The detection limit was 0.2 ppm, based on a 5 g sample.

Fungicide levels in young twigs were around 10 ppm, one week after each monthly treatment (Table 2) and then decreased to undetectable levels about two months later.

Captan and benomyl are compounds with a wide spectrum of fungitoxic activity. Their residues found in young twigs during the growth period, together with their biological activity, can explain the reduction of the epiphytic mycoflora observed in peach trees treated with these fungicides in a previous work.[12]

CONCLUSIONS

Levels of thiram, captan and benomyl in peach twigs can be determined by the proposed methods with enough sensitivity and reproducibility.

Thiram, usually applied at the end of winter, showed, one week after application, residue levels in old twigs around 50 ppm. Nevertheless, it was undetectable in young twigs grown afterwards.

Captan and benomyl, applied alone or all together during spring and summer, presented levels in young twigs between 5 to 20 ppm, a week after treatment,and then decreased to practically undetectable levels in about two months. Those residue levels can explain the reduction of epiphytic mycoflora previously observed in treated peach trees.

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