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Fate of the Fungicide Tolylfluanid in the Pear Cold Stored in Controlled or Non Controlled Atmosphere

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Tolylfluanid (N-dichlorofluoromethylthio-N',N'-dimethyl-N-p--tolylsulfamide; Euparen M) is a contact fungicide used preventively with success against Venturia spp. on apples and pears, with a secundary activity against powdery mildews and against red spider mites. It is used against Botrytis spp. and downy mildews of strawberry, lettuce and vineyards. It is used for the protection of apples and pears during the cold storage, especially against Botrytis which induces the rotting of pear during the cold storage. Dichlofluanid (N-dichlorofluoromethylthio-N',N'-dimethyl-N-phenylsulfamide; Euparen) has a chemical structure similar to the one of tolylfluanid, but without the 4-methyl aryl substituent. Both fungicides are used for the same applications.

In the foliage of the strawberry plant, dichlofluanid (A) is transformed into N,N-dimethyl-N'-phenylsulfamide (B; Vogeler and Niessen, 1967). Very little however has been published about the amounts of the residues of compounds A and B which are present in plant and fruit. In vitro, dichlofluanid is decomposed by alkaline aqueous hydrolysis into compound B (Vogeler and Niessen, 1967); however, the rate at which compound B is generated, and the stability of compound B toward consecutive hydrolysis have not been described. Photolysis of dichlofluanid generates several products among which is compound B (Clark and Watkins, 1978); in vitro tests against Botrytis cinerea showed that irradiation decreased the activity of dichlofluanid.

Dichlofluanid has a N-perhalogen-methylmercapto moiety, like the captan and folpet fungicides. The fungitoxic properties of captan were related to that trichloromethylthio moiety, as this last reacts with the sulfhydryl compounds (cysteine, glutathione...) in Saccharomyces, and simultaneously losses its fungitoxicity (Schuphan et al., 1981); the reaction pathway should involve thiophosgene as unstable intermediate. Compound B thus should not be fungitoxic.

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At our knowledge, about nothing has been published about the reactivity of tolylfluanid in vitro, and about its fate in biological medium, especially in plant and fruit. Fungicides are applied onto apple and pear before cold storage. This is done for the control of storage rots which are mainly due to Gloeosporium and Botrytis in apple, and mostly to Botrytis in pear. Very little has been published about the fate of fungicides in the fruit during cold storage. The disappearance of the residues of thiabendazole and benomyl in the pear during cold storage has been measured (Ben-Arie, 1975).

In the present work, we studied the fate of tolylfluanid in the pear during cold storage. Tolylfluanid has been sprayed several times on the pear trees during the 4 weeks which preceeded harvest. We first made some assays in vitro of hydrolysis and oxidation of tolylfluanid, in order to evaluate its chemical stability.

MATERIALS AND METHODS

The pears "Conference" were sprayed in the orchard (Fruit Research Station of Gorsem, Belgium) three times before harvest, with an emulsion of Euparen M 50WP (50 g% of tolylfluanid) in water (0.15 g% of Euparen M in water; 0.075 g% of tolylfluanid in water). At each treatment, the applied dose was 1.125 kg of tolylfluanid/ha. The first spray (10-8-1988, day-month-year) was made 33 days before harvest (12-9-1988); the second spray (22-8-1988) was made 21 days before harvest; the third spray (1-9-1988) was made 11 days before harvest.

A part of the pears was stored in a non controlled atmosphere at -0.5°C (State School for Horticulture, Vilvoorde, Belgium). Another part of the pears was stored in a controlled atmosphere (2% O_2, 0.7% CO_2, 97.3% N_2 at -0.5°C in a cold store at the auction Haspengouw (St Truiden, Belgium). For analysis, the samples of pears were taken out from the cold stores several times during the storage period.

T.l.c. was made with silicagel Merck 60F254 plates, 20x20 cm, 0.2 mm thick. The analyzed solution was applied as a band. The reference standards were applied on another part of the t.l.c. plate.

Tolylfluanid (1) and compound 2 were analyzed as such by g.l.c. by means of the Tracor 550 apparatus. Injection at 250°C, detection at 180°C. Glass column 1.8 m x 2 mm i.d., 5% SE30 on Gas Chrom Q 80-100 mesh, nitrogen as carrier gas at 40 ml/min, flame photometry detector (FPD) in the sulfur mode. Compound, column temperature, retention time: tolylfluanid, 210°C, 4.2 min; compound 2, 180°C, 3.1 min.

The i.r. spectra were recorded with the Perkin Elmer 297 spectrometer (KBr; cm^{-1}). ^1H n.m.r. spectra (δ , ppm/TMS) were recorded with the Varian XL200 spectrometer (200 MHz) in $CDCl_3$ containing tetramethylsilane as internal standard. The m.s. were recorded with the V.G. Micromass 7070F apparatus used in the electron impact mode at 70 eV (m/e, relative abundance, %). Several times, tolylfluanid and compound 2 present in the extracts from pear were analyzed by m.s.

Syntheses of the standards for analysis were made in the following ways. TOLYLFLUANID was isolated from the Euparen M formulation (50 g% of tolylfluanid). The mixture Euparen M (200 g) and chloroform (400 ml) was stirred (25 min, 20°C), filtered, the precipitate was washed with chloroform (100 ml), the filtrates were gathered, and washed with a saturated solution of NaCl in water. The chloroform solution was dried with sodium sulfate, concentrated in a vacuum rotavapor, giving 99 g of tolylfluanid. Recrystallization (methylene chloride+hexane, 1+1 ml/ml) gave tolylfluanid with a purity higher than 98%.
I.r.: 2960, 1520, 1480, 1375, 1280, 1220, 1165, 1040, 980, 965, 940, 890, 840, 820, 730.
^1H N.m.r.: 2.30 (s, 3H, $ArCH_3$); 2.70 (s, 6H, $N(CH_3)_2$); 7.17 (m, 4Harom).
M.s.: 346 (9, $M^+\cdot$); 348 (6); 350 (1); 239 (40, $M-SO_2N(CH_3)_2+H$); 241 (26); 243 (4); 214 (8, $M-SCCl_2F+H$); 215 (9); 181 (25, $CH_3C_6H_4NSN(CH_3)_2$); 137 (100, $CH_3C_6H_4NS$).

For the N',N'-DIMETHYL-N-p-TOLYLSULFAMIDE (2) synthesis, the mixture of dimethylsulfamoyl chloride (7.5 g), p-toluidine (11.2 g) and benzene (40 ml) was heated to reflux (2 hr, stirring), and filtered after cooling. Benzene (160 ml) was added, the mixture was washed successively with diluted solutions of HCl and Na_2CO_3 in water, and with a saturated solution of NaCl in water. The benzene solution was dried with sodium sulfate, and concentrated in a vacuum rotavapor. Crystallization of the residue (benzene+hexane) gave compound 2 (yield =89%).
I.r.: 3300, 2950, 1620, 1600, 1530, 1480, 1410, 1340, 1315, 1290, 1235, 1220, 1155, 975, 930, 830, 730.
^1H N.m.r.: 2.27 (s, 3H, $ArCH_3$); 2.80 (s, 6H, $N(CH_3)_2$); 7.03 (s, 4H arom.).
M.s.: 214 (50, $M^+\cdot$); 152 (4, $M-SO_2+2H$); 135 (6, $M-NHSO_2$), 106 (100, $CH_3C_6H_4NH$).

The reactivities of tolylfluanid (1) and of compound 2 toward in vitro alkaline hydrolysis and oxidation, were studied. For the TOLYLFLUANID reactivity study, the mixture of tolylfluanid (2 g), water (50 ml), and KOH (2 g) was refluxed with stirring (10 min). Reaction was made either under a nitrogen current (after having bubbled nitrogen in the mixture before to start heating), either under a current of air. The cooled mixture was filtered, giving precipitate a and the filtrate; this was

extracted with ethyl acetate (300 ml), the ethyl acetate solution was dried with sodium sulfate, and concentrated to dryness in a vacuum evaporator (30°C), giving product b. Products a and b were purified separately by column chromatography on silica-gel (elution successively with toluene+hexane 1+2 ml/ml, and by ethyl acetate+hexane 1+2), and analyzed by g.l.c. When heating to reflux was made under a nitrogen atmosphere, the sum of the products a and b was made up of 0.8 g of tolylfluanid (40%) and 0.7 g of N',N'-dimethyl-N-p-tolylsulfamide (2; 57%). When the hydrolysis reaction was made under a current of air, there was no more tolylfluanid, but 1.15 g of compound 2 (93%) were formed. The same products were generated when the hydrolysis was made in 2N HCl in water with heating to reflux.

For the N',N'-DIMETHYL-N-p-TOLYLSULFAMIDE (2) reactivity study, the mixture of compound 2 (2 g), water (50 ml) and KOH (2 g) was heated to reflux (stirring, 2 hr). The cooled mixture was saturated with NaCl, extracted with ethyl acetate, the ethyl acetate solution was dried with sodium sulfate, and concentrated to dryness in a vacuum rotavapor (30°C). The residue was analyzed as indicated above for the tolylfluanid reactivity study, and contained 1.56 g (78%) of compound 2.

For the analysis of the peel of the pears, the pears were peeled (peelings 3 to 4 mm thick). An aliquot of peelings (25 g) from 15 pears was ground in a fruit juice extractor Braun. The juice and the pulp from extraction were gathered; the apparatus was rinsed with ethanol (40 ml), the ethanol was added to the mixture of juice and pulp, ethyl acetate (200 ml) was added to the whole, the mixture was heated to reflux (10 min, stirring), and filtered after cooling. The filtrate was poured onto a column (3.5x28 cm) containing anhydrous sodium sulfate (70 g), and supplementary ethyl acetate (100 ml) was run onto the column. The eluates were gathered, concentrated to 40 ml in a vacuum rotavapor (30°C), and to 1 ml by means of a current of nitrogen. The extract was applied onto a t.l.c. plate. Elution by means of toluene gave the band 1 (Rf=0.62) containing tolylfluanid, and the band 2 (Rf=0.19) containing compound 2. The band 1 was scraped off separately, extracted with ethyl acetate in a small glass column, the ethyl acetate solution was concentrated to 1 ml by means of a nitrogen current, and tolylfluanid was analyzed in the extract by means of g.l.c. and, occasionally, by m.s.

In the same way, band 2 (containing compound 2) was scraped off, extracted with ethyl acetate, the extract was reduced to 1 ml, and applied onto a second t.l.c. plate; elution with ethyl acetate+hexane 1+2 ml/ml gave the band 3 (Rf=0.82) which contained compound 2. The band 3 was separated, extracted, and the extract was analyzed by g.l.c. and, occasionally, by m.s. for the analysis of compound 2.

The pulp of the pear was cut into small pieces, these were ground in the fruit juice extractor, and analyzed in the same way as the peel.

At the level of 0.2 mg/kg fresh weight in the peel and in the pulp, the recoveries of tolylfluanid and of compound 2 were 87-93% and 82-89% respectively. The analytical limit of sensitivity was 0.02 mg/kg of fresh weight for both these compounds in the peel and in the pulp.

RESULTS AND DISCUSSION

The chemical stabilities of tolylfluanid and of compound 2 were first evaluated by making in vitro hydrolysis and oxidation assays. In water containing 4 g% of KOH, and with heating to reflux during 10 min, tolylfluanid was rapidly transformed into compound 2. The reaction occurred both in the absence of oxygen (nitrogen atmosphere; hydrolysis alone), and in its presence (hydrolysis+oxidation). Oxygen accelerated the transformation of tolylfluanid into compound 2. The reaction products originating from the $SCCl_2F$ moiety have not been identified. The following hypothesis however explains the acceleration, due to oxygen, of the hydrolysis. In the presence of oxygen, the non isolated compound 3 (carrying two geminal SO_2 groups on the nitrogen bound to the toluene nucleus) should be formed (Figure 1). The nucleophilic attack of the S atom by the OH^- is faster with compound 3 than with tolylfluanid. The hydrolysis of compound 3 thus should be faster than the one of tolylfluanid. In the absence of oxygen, the hydrolysis should occur along pathway a; in the presence of oxygen, it should occur along the pathways b+c which, in the total, are faster than pathway a.

Compound 2 has a good stability toward hydrolysis. In 2N HCl in water, and with heating to reflux, the reactivities of both tolylfluanid and compound 2 were similar to the reactivities observed in the aqueous alkaline solution.

IN THE PEEL OF THE PEAR, both tolylfluanid and compound 2 were observed. In the fruit tissues, tolylfluanid was transformed during the cold storage probably by both hydrolysis and oxidation biochemical reactions. This is suggested by the in vitro reactivity assays made with tolylfluanid. The biochemical oxidation perhaps should also correspond to the decomposition of tolylfluanid into compound 2, which was faster in non controlled atmosphere, than in controlled atmosphere (low oxygen concentrations).

During storage in controlled atmosphere, the tolylfluanid concentration in the peel roughly did not change during the first 3.5 months of storage (till the end of December 1988); after that, the biodegradation accelerated; that acceleration increa-

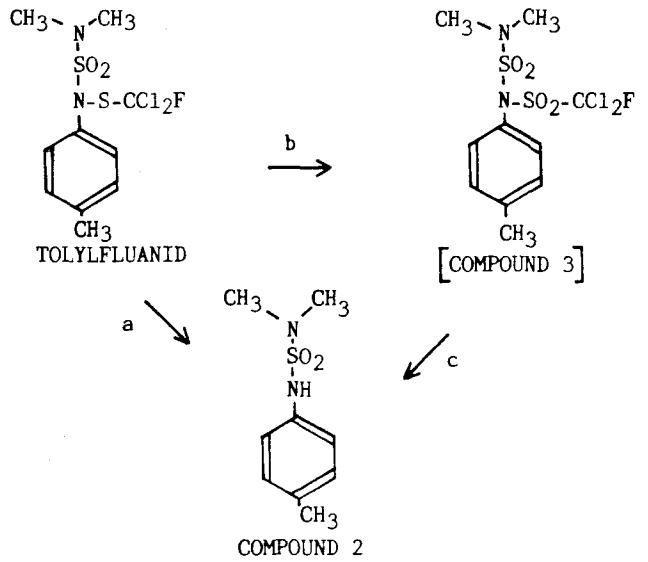

Figure 1. Hydrolysis and oxidation degradation of tolylfluanid.

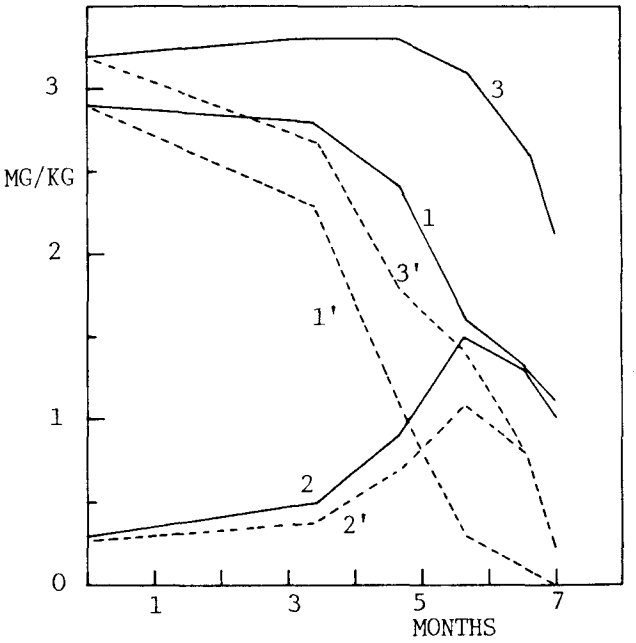

Figure 2. Evolution of the concentrations (mg/kg fresh weight) of tolylfluanid (1; curve 1), compound 2 (2), and of the sum tolylfluanid+compound 2 (3) in the peel of the pear during cold storage in controlled atmosphere (full lines, non primed figures), or in non controlled atmosphere (dotted lines, primed figures).

TABLE 1. Concentrations of tolylfluanid (1) and compound 2 in the peel and the pulp of the pears during their cold storage in controlled atmosphere (CA) or in non controlled atmosphere (NCA).

Date a	Days in the cold	Concentrations of tolylfluanid (1) and compound 2 (as equivalents of tolylfluanid) in the peel and the pulp (mg/kg fresh weight)b							
		Peel				Pulp			
		CA		NCA		CA		NCA	
		1	2	1	2	1	2	1	2
12-9-1988	0	2.9	0.3	2.9	0.3	0.1	n.d.	0.1	n.d.
20-12-1988	99	2.8	0.5	2.3	0.4	0.1	0.1	0.1	0.1
1-2-1989	142	2.4	0.9	1.1	0.7	n.d.	n.d.	n.d.	n.d.
2-3-1989	171	1.6	1.5	0.3	1.1	n.d.	n.d.	n.d.	n.d.
28-3-1989	197	1.3	1.3	n.d.	0.8	n.d.	n.d.	n.d.	n.d.
10-4-1989	210	1.1	1.0	n.d.	0.2	n.d.	n.d.	n.d.	n.d.

a. Day-month-year. At 12-9-1988, harvest and introduction in the cold rooms. b. 1: tolylfluanid; 2: N',N'-dimethyl-N-p-tolylsulfamide. Means of 4 repetitions. n.d.=non detected.

sed with the time of cold storage (Table 1, Figure 2). This probably corresponded to the fruit evolution during cold storage. After 4 months of cold storage -and during which time the fruit biochemical activity was completely reduced- that biochemical activity revived; to that corresponded the biodegradation of tolylfluanid. The peel concentration of compound 2 increased during cold storage, attained the maximum of 1.5 mg/kg, and then decreased.

The same process occurred in the non controlled atmosphere, but much faster; to that corresponded a faster tolylfluanid biodegradation. The maximum peel concentration of compound 2 was lower (1.1 mg/kg) when the fruit was cold stored in a non controlled atmosphere, than when it was stored in a controlled atmosphere (1.5 mg/kg). This indicated that compound 2 in his turn was biodegraded in the peel, into non identified compounds.

In controlled atmosphere, the sum tolylfluanid+compound 2 did not change very much during the first 5 months of cold storage; after that, that sum decreased fastly. This also could correspond to the starting again of the fruit biochemical activities during cold storage. To that corresponded the slow ripening during cold storage; that slow ripening (slow because braked by the cold) was due to the increase of the fruit respiratory activity which corresponded to the climacterium.

In non controlled atmosphere, the decrease of the sum tolylfluanid+compound 2 was already pronounced during the first 3.5 months of cold storage; after that, the decrease of that sum was still faster than in controlled atmosphere during the same period of time. This corresponded to the fruit biochemical activity which was greater during cold storage in non controlled atmosphere, than in controlled atmosphere. When the pears were taken out of the cold rooms in April 1989, the pears stored in non controlled atmosphere were yellow and ripen, and good for direct consumption; at the opposite, at the same moment the pears stored in controlled atmosphere were still green, and not good for eating.

In the pulp of the pear, the residues of tolylfluanid and of its metabolite compound 2 were very low. These residues were only observed after harvest, and during the first 3 months of cold storage. In February 1989, no more residues were observed in the pulp of the pear stored in controlled or in non controlled atmosphere.

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