Residues of Quintozene, Hexachlorobenzene, Dichloran and Pentachloroaniline in Soil and Lettuce

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The use of quintozene (PCNB, pentachloronitrobenzene) as a fungicide in lettuce growing is widespread in Belgium. In a residue survey carried out in 1973, about half of the samples analysed contained residues of this compound (DEJONCKHEERE et al. 1974a). The amounts found were in all cases below the Belgian and Dutch tolerance of 3 ppm, although sometimes higher than the German tolerance of 0.3 ppm.

It was also found that a number of samples contained quintozene residues although it was certain that the crop had not been treated with this fungicide. However quintozene was found in the soil as a result of treatments in the previous years, indicating that this could be a source of quintozene residues in lettuce.

With the object of confirming this observation and investigating to what extent quintozene might be taken up from the soil, residue analyses were carried out on lettuce grown on soil treated with quintozene 18 months before harvesting and after applying two dosage rates and a mixture of quintozene and dichloran on the same soil just before and two weeks after planting.

Apart from quintozene and dichloran, hexachlorobenzene (HCB), pentachloroaniline (PCA) and pentachlorothioanisole (PCTA) residues were determined in soil and in lettuce plants.

HCB residues occur because of the presence of between 2.28 % and 2.36 % HCB in the technical grade quintozene applied. HCB is normally present as an impurity in technical grade quintozene in amounts varying between 1 % and 6.2 % (CASANOVA and DUBROCA, 1972) as a result of the pathway followed in industrial synthesis. By appropriate changes in this procedure the HCB content may be reduced to less than 0.1 %. This however results in a higher percentage of pentachlorobenzene (QCB) as an impurity varying between 0.5 and 2 %. In the applied commercial formulations QCB is also present but in much smaller amounts, normally less than 0.1 %, not detectable as a residue with the analytical methods used in the present work.

Pentachloroaniline (PCA) and pentachlorothioanisole (PCTA) are metabolites of quintozene mainly produced by microorganisms in the soil. Especially PCA is normally present in plants where quintozene residues are found (GORBACH and WAGNER, 1967; KUCHAR et al, 1969; KO and FARLEY, 1969; WANG and BROADBENT, 1973; DE VOS et al, 1974).

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Experimental

Variety : Vera Pil (from resistant KLOCK)
Date of sowing : 21.11.73
Date of planting : 14.01.74
Treatments : quintozene and dichloran were applied in the glasshouse on a soil where quintozene had been applied in september 1972 (40 g Brassicol conc. (50 %)/are : 20 g quintozene/are).

Experiment 1 : Untreated
Experiment 2 : 40 g Brassicol conc. (50 %)/are = 20 g quintozene on 14.1.74 as a pre-planting soil treatment
Experiment 3 : dosage as 2 on 28.1.74 as a post-planting treatment on the crop
Experiment 4 : 400 g Brassicol conc. (50 %)/are = 200 g quintozene on 14.1.74 as a pre-planting soil treatment.
Experiment 5 : dosage as 4 on 28.1.74 as a post-planting treatment on the crop
Experiment 6 : 1.2 kg Combisan (dust) = (8 % dichloran and 9.3 % quintozene)/are = 96 g dichloran and 116 g quintozene on 14.1.74 as a pre-planting soil treatment.
Experiment 7 : dosage as 6 on 28.1.74 as a post planting treatment on the crop.

All treatments were carried out in triplicate on randomized plots in the same glasshouse.

During the growing season three other fungicide treatments were carried out.

24.1.74 - thiram 1.6 kg/are Pomasol (80 %)
7.2.74 - thiram 1.6 kg/are Pomasol (80 %)
27.2.74 - thiram 1.0 kg/are Pomasol (80 %)

Date and amounts of artificial rainfall per m^2

25.1.74	20 ml	19.3.74	70 ml
30.1.74	39 ml	25.3.74	43 ml
9.2.74	17 ml	1.4.74	69 ml
20.2.74	79 ml	5.4.74	94 ml
28.2.74	35 ml	8.4.74	60 ml
8.4.74	93 ml		

Extraction and analysis

Each sample consisted of 5 heads of lettuce. The four outer leaves of all heads (shown as Ou in the tables) and the rest of the plant (In) were analysed separately. Total residue in ppm of fresh plant material was then obtained by calculation from the obtained residue values and the weight of the two sample fractions.

This procedure is followed because earlier work (DEJONCKHEERE et al

1974a,b,c) had shown that in lettuce 70 to 90 % of the total residue is found on these four outer, oldest leaves when the last treatment had been carried out three to four weeks before harvesting. This is mainly due to the very rapid increase in weight of the lettuce plants in this period, resulting from the development of numerous young leaves having had no direct contact with the earlier applied fungicide. Uptake from the soil by these young leaves is also restricted because of the short period of time elapsing between their appearance and harvest. Following this procedure is considered necessary for obtaining acceptable reproductibility of results.

For soil analysis, two 50 g subsamples were taken from a 1 kg soil sample, resulting from about 20 cores per plot taken with a hand drill of 2.5 cm diameter at a depth of 20 cm.

Reagents and apparatus

Petroleum ether, freshly distilled
Acetone , freshly distilled
Sodium sulphate, anhydrous
Sodium chloride, p.a.
Florisil , activated at 130°C during 10 hours.

Varian Aerograph gaschromatographs equipped with electron capture detector and glass collumns filled with 2 % OV 225 and 3 % of a mixture of OV 17 and OV 210 (3-22) on Gas Chrom Q (fig. 1).

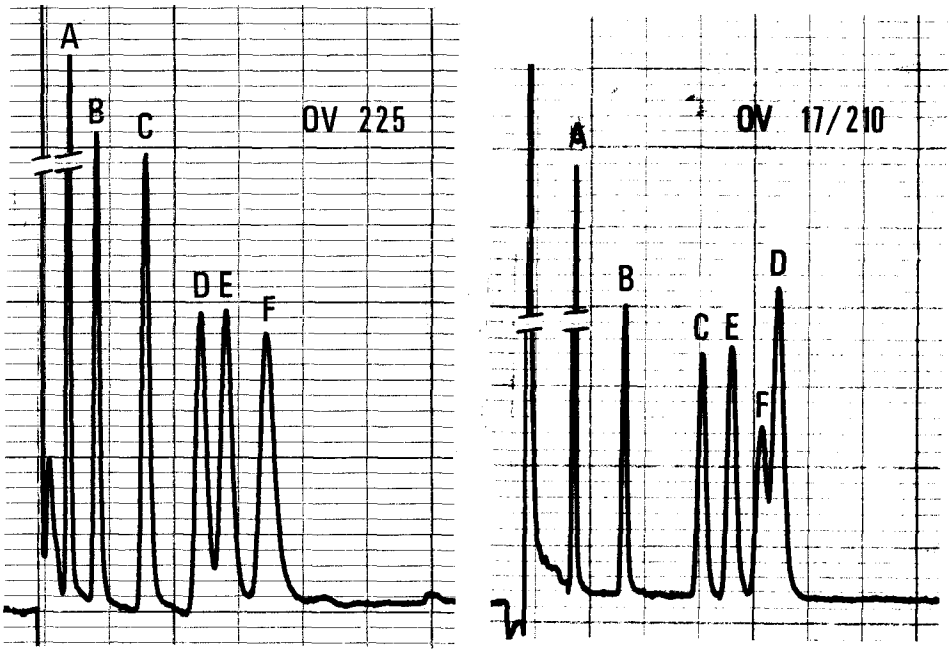

Fig. 1 - Chromatograms of QCB (A), HCB (B), PCNB (C), PCTA (D) PCA (E) and Dichloran (F).

Extraction and clean-up

100 g of finely sliced lettuce leaves or 50 g soil is blended for 3 minutes with 200 ml of a 1:1 petroleum ether/acetone mixture. After filtering trough filterpaper using a Buchner funnel, the residue is rinsed with 50 ml of the same solvent mixture.

The solution is then transferred to a 1 litre separating funnel and shakentwice with 200 ml of water and 25 ml of a saturated NaCl solution.

The petroleum ether layer is dried over anhydrous Na_2SO_4. The resulting solution may be used as such,further dilution being occasionally necessary for determining quintozene, PCA and dichloran.

For HCB analysis,as very small amounts are involved,the solution is concentrated in a Rotavapor after shaking with a small amount of florisil.

Analysis

The contents of quintozene, HCB, PCA, PCTA and dichloran were determined by gaschromatography, concentrations were measured by comparing peak heights of the unknown sample with those of known concentrations injected at regular intervals.

Results

(All results are expressed in ppm unless otherwise stated)

Residues in the soil

Soil analyses were carried out 7 weeks before the first lettuce samples were analysed (6.2.74) and later on the same dates as the crop was sampled (25.3.74 and 8.4.74). Each analysis was carried out in duplicate for each of the three plots relating to experiments 1 to 7. Thus for each treatment six analyses were carried out at each sampling date.

Table I shows the average results for quintozene, HCB and dichloran per experiment for each sampling date and for HCB as a percentage of the quintozene present.

Residues in the lettuce crop

The crop was sampled and analysed on 25.3.74 and 8.4.74. These dates are related to early or late harvesting in practice. Each replicate plot was sampled and analysed separately and the outer (Ou) and inner (In) leaves were analysed separately. The average of these results, the total calculated residue in fresh leaves and the mean weight per head are given in table II.

The soil/crop relation for quintozene, HCB and dichloran are shown in table III.

TABLE I

Average content of quintozene (PCNB), hexachlorobenzene (HCB) and dichloran in the soil

Mean of the three plots and analyses A and B on three dates

		5.2.74	25.3.74	8.4.74
Exp. 1	PCNB	0.15	0.13	0.11
	HCB	0.0085	0.007	0.006
	% HCB	5.7	5.4	5.4
Exp. 2	PCNB	0.80	0.44	0.44
	HCB	0.031	0.019	0.019
	% HCB	3.9	4.3	4.3
Exp. 3	PCNB	0.84	0.49	0.32
	HCB	0.028	0.015	0.010
	% HCB	3.3	3.1	3.1
Exp. 4	PCNB	7.55	6.03	6.18
	HCB	0.22	0.19	0.17
	% HCB	2.9	3.1	2.7
Exp. 5	PCNB	7.27	6.28	4.90
	HCB	0.19	0.17	0.14
	% HCB	2.6	2.6	2.9
Exp. 6	PCNB	3.85	2.96	2.23
	HCB	0.096	0.073	0.073
	% HCB	2.5	2.4	3.4
	dichloran	2.62	2.25	2
Exp. 7	PCNB	3.88	2.45	2.35
	HCB	0.133	0.075	0.068
	% HCB	3.3	3.1	3
	dichloran	2.42	1.72	1.82

TABLE II

Average content of quintozene (PCNB), hexachlorobenzene (HCB) dichloran and pentachloroaniline (PCA) in the lettuce (fresh matter)

Mean of the three plots of each experiment on two dates

		PCNB			HCB			PCA			Dichloran			Weight per head (g)
		In	Ou	Tot	In	Ou	Tot	In	Ou	Tot	In	Ou	Tot	
Exp. 1	25.3.74	0.19	0.86	0.37	0.008	0.018	0.011	0.10	0.69	0.25				154
	8.4.74	0.06	0.32	0.11			<0.005	0.08	0.37	0.14				333
Exp. 2	25.3.74	0.26	1.06	0.49	0.013	0.018	0.015	0.18	0.81	0.36				153
	8.4.74	0.13	0.50	0.19			<0.005	0.13	0.50	0.19				321
Exp. 3	25.3.74	0.28	1.41	0.56	0.012	0.020	0.014	0.18	1.01	0.39				148
	8.4.74	0.11	0.39	0.16			<0.005	0.11	0.48	0.19				321
Exp. 4	25.3.74	1.61	6.33	2.67	0.075	0.20	0.11	0.64	2.01	0.99				142
	8.4.74	0.34	3.06	0.89	0.014	0.08	0.032	0.26	1.15	0.44				319
Exp. 5	25.3.74	2.31	9.83	4.19	0.09	0.17	0.12	0.78	2.90	1.52				119
	8.4.74	0.62	5.47	1.56	0.018	0.15	0.045	0.30	1.87	0.60				280
Exp. 6	25.3.74	0.48	2.27	0.92	0.015	0.061	0.026	0.18	1.01	0.38	0.067	0.313	0.130	172
	8.4.74	0.25	0.94	0.36	0.004	0.030	0.009	0.14	0.60	0.23	0.04	0.233	0.086	355
Exp. 7	25.3.74	0.65	3.2	1.28	0.019	0.07	0.032	0.27	1.21	0.48	0.14	0.84	0.30	170
	8.4.74	0.35	2.37	0.73	0.011	0.047	0.017	0.18	0.76	0.31	0.08	0.45	0.15	340

TABLE III

Soil/crop relation of quintozene, HCB and dichloran

	Quintozene				HCB			Dichloran		
	Soil	Lettuce		Relative content soil-lettuce	Soil	Lettuce	Relative content soil-lettuce	Soil	Lettuce	Relative content soil-lettuce
Exp. 1	0.12	A	0.37	3	0.007	0.011	1.60			
		B	0.11	0.92		<0.005	<0.70			
Exp. 2	0.44	A	0.49	1.11	0.019	0.015	0.79			
		B	0.19	0.43		<0.005	<0.26			
Exp. 3	0.40	A	0.56	1.4	0.013	0.014	1.08			
		B	0.16	0.4		<0.005	<0.38			
Exp. 4	6.1	A	2.67	0.44	0.18	0.11	0.61			
		B	0.89	0.15		0.032	0.17			
Exp. 5	5.6	A	4.19	0.75	0.16	0.12	0.75			
		B	1.56	0.28		0.045	0.28			
Exp. 6	2.6	A	0.92	0.35	0.073	0.026	0.35	2.13	0.13	0.06
		B	0.36	0.14		0.009	0.12		0.086	0.04
Exp. 7	2.4	A	1.28	0.53	0.079	0.032	0.40	1.77	0.30	0.17
		B	0.73	0.30		0.017	0.21		0.15	0.08

Soil : mean of samples taken on 25.3.74 and 8.4.74
Lettuce : A - early harvest B - late harvest (two weeks later)

Discussion and conclusions

The results show that following a quintozene treatment at 20 g/are 18 months prior to harvesting a lettuce crop, the plants contain on the average 0.37 ppm on the first sampling date (early harvest) and 0.11 ppm two weeks later (late harvest) on a fresh leaf basis, while the soil contains 0.12 ppm of quintozene on the average.

Treating the same soil with the same dose (20 g/are) just before or two weeks after planting increases the soil residue at the time of harvesting on the average to 0.44 and 0.40 ppm respectively and in the lettuce plants to 0.49 and 0.57 ppm at the first sampling date and 0.19 and 0.16 ppm two weeks later. The treatment with a quintozene dose of 200 g/are resulted on the average in residues of 6.1 and 5.6 ppm in the soil and 2.67 and 4.19 ppm in the lettuce on the first sampling date and 0.89 and 1.56 ppm two weeks later.

The results of experiment 1 clearly show that application of quintozene on preceding crops leads to quintozene residues in crops grown on the same soil the following year. This is in agreement with the observations of CASANOVA and DUBROCA (1972) and SMELT and LEISTRA (1974). From the relatively low increase of the residue content following a new treatment at the rate of 20 g/are, it would seem that quintozene which has been present in the soil for some time is taken up more readily by the plant than the freshly applied chemical. The sharp decrease of these values during the two week period separating the two sampling dates may be explained by the increase (almost doubling) in weight of the plants during this time, the dying-back of the outer leaves containing the highest residues and the formation of PCA from quintozene in the plant, indicated by the relatively slight decrease of this metabolite during the same period.

No measurable residues of PCA and PCTA could be detected in the soil samples at the date of harvest.

This is in contradiction with some of our recent findings and the results of other experiments (KO and FARLEY, 1969; WANG and BROADBENT, 1973; DE VOS et al, 1974; DEJONCKHEERE et al 1974d), which indicate that in soils which contain quintozene these metabolites are also present.

A possible explanation is that in this case the dose of the treatment in 1972 was very low and the time between the new treatment and the sampling date was too short for the formation of measurable quantities of PCA and PCTA (.)

As residues of PCA were found in the plant this may indicate the formation of this metabolite in lettuce and/or a rapid uptake from the soil.

(.) *Analysis of the same soil six months after harvest showed the presence of small amounts of PCA and PCTA.*

Residues of HCB in lettuce are either less than 0.005 ppm or about 2 to 3 % of the quintozene content. This may indicate a slower uptake of HCB from the soil, the more so as quintozene is partially metabolized to PCA.

Application of the quintozene - dichloran mixture results in comparable residue levels of quintozene and HCB residues in the soil. The average dichloran content of the soil at the time of harvest is 2.12 ppm for the pre-planting treatment and 1.77 ppm for the application two weeks after planting. After this treatment the residue levels of quintozene in the lettuce are relatively lower than after treating with quintozene alone. Applying quintozene (116 g) mixed with dichloran (96 g) leads to 0.92 ppm in the crop following a pre-planting treatment and 1.28 ppm post planting. These figures are respectively 2.67 and 4.19 ppm when a 200 g quintozene dose is applied.

The results obtained allow the following clonclusions.

1. Although quintozene is not classified as a systemic fungicide, it is readily taken up by the lettuce and other plants through the root system.

2. Quintozene is metabolized to pentachloroaniline (PCA) and pentachlorothioanisole (PCTA). PCA is present in the plant in measurable amounts. From other experiments it can be concluded that both are normally present in the soil some time after the application of quintozene.

3. Hexachlorobenzene is also taken up from the soil by the lettuce plant, and the resulting quintozene/HCB ratio is almost identical in the plant as in the soil.

4. The uptake of dichloran follows a similar pattern as that of quintozene.

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Summary

The results are described of the residue determination of quintozene, hexachlorobenzene, dichloran and pentachloroaniline in soil and lettuce after application of different quantities of quintozene just before and two weeks after planting. The results indicate that quintozene, hexachlorobenzene and dichloran are readily taken up by lettuce from the soil and that quintozene is metabolised to pentachloroaniline resulting in residues of these compounds in lettuce.

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