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Residues of chlormequat (CCC) in fruits and other parts of tomato plants after treating the seedlings with ^{14}C-CCC

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Abstract

CCC remaining in tomato plants after treating the seedlings with a solution of 125 mg/l CCC to prevent their excessive growth has been studied in experiment conducted for two years. When seedling had been treated twice with CCC, the tomatoes of the first crop from these plants contained 0.09 mg CCC/kg fresh fruit. The amount of residual CCC decreased with each further crop. The last crop contained only 0.02 mg CCC/kg fresh fruit. The amount of CCC in the remains of leaves and stems at the end of the vegetation period was similar to that in the fruit of the first crop, however, the amount of CCC in the remains of the roots was several times larger than in the fruit. CCC which had been added directly to compost soil was quickly degraded.

INTRODUCTION

During the last few years, a number of publications have appeared dealing with the possibility of using 2-chloroethyl-trimethyloamonium (chlormequat, CCC) in tomato culture (Knavel, 1969; Weichold et al., 1970; Ledovskii, 1974, 1977; Kratochvilova and Frydrych, 1977; Shulgina, 1977; Pisarczyk and Splittstoesser, 1979; Pill et al., 1979).

CCC causes shortening of the internodes and stimulation of root system growth of tomato seedlings (Wittwer and Tolbert, 1960; Ledovskii, 1977), which bears upon the increased resistance of young plants to drought and often on a decidedly larger early crop, sometimes also on a larger market crop. Larger crops from CCC treated plants are also due to better taking of seedlings after they are transplanted (Borkowski, 1976). This is especially evident when a mechanical planter is used (Borkowski and Jagoda, 1977).

CCC is, however, toxic to animals and man, and on the basis of the DL_{50} index (50-500 mg/kg) has been assigned to the II or III class of toxic substances (Blaim, 1969 and Müller, 1972). It is therefore very important to know how much CCC is found in the fruit of plants treated with it. There are few papers dealing with residual CCC in tomatoes. Studies done by Müller show very large amounts of residual CCC – from 2.7 to 25 ppm in fresh fruit, however, he used relatively high doses of CCC: 25-250 mg CCC/plant. The dose recommended by Borkowski (1976) and Borkowski and Jagoda (1977) is several times lower – 5 mg CCC/plant in two spray treatments with a solution of 125 mg CCC/l; a much lower residual amount of CCC in fruit should therefore be expected. In the method used by Borkowski (1976) seedlings in the hotbed were sprinkled with about 20 ml of solution per/plant using a watering--can fitted with a fine sieve or using a sprayer. Part of the solution was absorbed directly by the soil. The plants were sprinkled twice – once at the 4 leaf stage and then again about 10 days later.

In studies conducted by Drygas (1973) on the amount of residual CCC in tomato fruits using thin-layer chromatography and in our own studies using a colorimetric method, the results obtained were below or on the limit of detectability. The aim of this study was to apply an isotope method to detect residual CCC. The use of ^{14}C-CCC enables detection of 10^{-4} µg CCC.

MATERIALS AND METHODS

The isotope method to detect residual CCC was used in studies conducted in 1976 and 1978 on a determinant cultivar 'Venture' widely cultured in Poland. The experiments were conducted in the greenhouse up until the seedlings were produced and treated (for details see Table 1). The temperature in the greenhouse during in the night ranged from 16 to 19°C, during the daytime – 19-23°C, in the sun at noon, to 30°C.

CCC whose methyl group was labelled with C-14 (produced by the Institute of Nuclear Research, Świerk) was used in these studies.

The stock solution of 250 mg CCC/l contained non-radioactive CCC and ^{14}C-CCC at a ratio of 5.0:1.0. In some experiments CCC at a concentration of 125 mg/l was used, obtained by dilution of the stock solution.

The plants were treated with radioactive ^{14}C-CCC (see Borkowski, 1976; Borkowski and Jagoda, 1977) twice with an interval of 7-10 days between treatments. In each case 20 ml of CCC solution at a concentration of 125 or 250 mg/l per plant were used. The shoots were either submerged in the CCC solution or it was also added to the soil. It had been previously found that the volume of CCC remaining on the shoot after it had been submerged was ~ 2 ml during the first treatment and ~ 5 ml during the second in 1976, and ~ 3,5 and ~ 6,5 ml, respectively in 1978. The remainder of the 20 ml portion was added to

Table 1

Calendar of procedure

Type of procedure	Dates 1976	1978
Sowing (S) and picking (p) to 0.9 l. pots	S:22.III, p:12.IV	S:11.IV, p:26.IV
Fertilizing with amonium nitrate (1%)	14.V	17.V, 5.VI
First and second treatment with ^{14}C-CCC	30.IV, 6.V	22.V, 27.V
Transplantation to vases*	17.V	8.VI
Fertilizing with nitrogen: 2 g/vase in 1978, 8 g/vase in 1976	31.V	12.VI
Fertilizing with chalk 10 g/vase	19.VI	3.VIII
Fertilizing with "Azofoska"** 7 g/vase in 1978 and 10 g/vase in 1976	5.VII	29.VI, 4.VIII
Control of aphids and white fly with Anthio, Pirimor, Actelic	2.VII	29.V, 15.VI 13.VII, 28.VII
First and last application of Betokson to stimulate fruit setting	28.V, 26.VI	12.VI, 3.VIII
First and last crop	25.VII, 8.IX	7.VIII, 26.IX

* – flowering began after transplanting to vases; ** – 10.5% N; 10.5% P_2O_5; 15.5% K_2O; 1.1% MgO, and about 0.1% B; 0.1% Mn; 0.1% Cu; 0.1% Zn; 0.1% Co.

the soil. In 1976 the experiment was comprised of three variants, described below:

(1) ~ 5 mg CCC/plant, from a solution of 125 mg CCC/l

(2) ~ 10 mg CCC/plant, from a solution of 250 mg CCC/l

(3) ~ 10 mg CCC/plant, from a solution of 250 mg CCC/l

In all cases the treatment was carried out as described above. Seedlings from variants (1) and (2) were transplanted after removal of soil from their roots by gentle shaking, seedlings from variant (3) were planted with the adjoining soil. In 1978, the following variants were employed:

(1) 10 mg CCC/plant, from a solution of 250 mg CCC/l }
(2) 5 mg CCC/plant, from a solution of 125 mg CCC/l } treated as described

(3) 2.4 mg CCC/plant, from a solution of 250 mg CCC/l } treated only by submerging in solution

All of the seedlings were planted without removal of soil from their roots.

There were 5 (1976) or 6 (1978) plants in each variant. A few days after treatment with radioactive CCC, the seedlings were transplanted from pots to vases filled with compost soil and placed outside the hotbed. The plants were propped with small pickets, and not trimmed. They were watered daily on sunny days, as necessary during cloudy weather. Developed flowers were treated with an auxin preparation (Betokson) every 6-8 days (Table 1) in order to obtain better fruit development (K ę p k o w a, 1959, 1968). Ripe tomatoes were gathered and weighed every few days from plants from each variant. The tomatoes were then sorted into 4 categories: healthy weighing $\geqslant$ 40 g, healthy weighing 20-39 g, healthy weighing $<$ 20 g, diseased, regardless of size.

The amount of residual CCC was determind in fruits from 4 (1976) and 7 (1978) consecutive crops from plants of all three variants. Leaves, stems and roots were also tested at the end of the vegetation period.

Radioactivity was determined with the following instruments: liquid scintillation counter SL-40; ZR-16 counter (produced by Polon — Warsaw) and Geiger-Müller counter. The type of counter used is noted in the Tables. 50 mg of dry, finely ground sample were used to determine radioactivity with the ZR-16 and Geiger-Müller counters. Each sample was taken in duplicate, counted 2-3 times for 10 minutes. Purified sample of CCC were prepared for liquid scintillation as described by J u n g and H e n j e s (1969). This method allowed possible CCC metabolites to be detected. The following liquid scintillator was used: 7 g PPO (2,5-diphenyloxazole), 100 mg POPOP (1,4-bis [2/5-phenyloxazolyl]--benzene), 100 g naphtalene in 1 liter of dioxane.

Studies on the degradation of ^{14}C-CCC in moist compost soil were carried out between June and September, 1976. Soil containing ^{14}C-CCC was placed under airtight bell-glasses, where liberated $^{14}CO_2$ was absorbed by a solution of KOH. The radioactivity of the KOH solution containing $^{14}CO_2$ was determined in a liquid scintillation counter. The temperature during these studies varied from 20 to 28°C and was the lowest in September.

The results presented in Table 2 were worked out with the method of analysis of variance. With Student's t-test for significance.

RESULTS

The plants treated with CCC were twice as small as control plants in 1976, and about 33% smaller in 1978 (Table 2). In spite of good condition (watering, fertilizing) the amount of tomatoes obtained from each plant in both experimental years is below 2 kg and is lower than expected (up to 4 kg/plant). This can be explained by unfavorable weather conditions (1978) and *Pyrenocheta lycopersici* on roots (1976) which had a great influence on the final crops and caused premature wilting of the plants. In 1978, in order to prevent this, the plants were

Table 2

Effect of CCC on the growth and size of crops of tomato plants in vases

Treatment			Height of seedling at transplantation (cm)		Crop – in kg/vase			
					total		marketable	
concentration of CCC	1976	1978	1976	1978	1976	1978	1976	1978
						ns		ns
Control	S–	S+	32.5c	41.8c	1.82b	1.25	1.78c	1.15
125 mg/l	S–	S+	17.3b	28.8b	1.45ab	1.24	1.37ab	1.10
250 mg/l	S–	S+	14.7a	24.7a	1.23a	1.31	1.10a	1.07
250 mg/l	S+	AT	13.7a	27.3ab	1.76b	1.35	1.65bc	1.11

S– – soil shaken off roots before planting; S+ – plants transplanted along with soil attached to roots; AT – only shoots treated with CCC; ns – not significant differences.
Number marked with the same letter do not differ significantly.

treated with Topsin and Dithane. The small crops are probably the cause of the higher than expected radioactivity of the fruit, leaves and stems.

In both years the highest level of radioactivity of fruits was found in the first crop (Tables 3, 4, 5). The radioactivity of the fruit from the following crops gradually decreased and was the lowest in the latest crops.

It is interesting to note, that in both experimental series, the variant which would be recommended for practical use (submerging the shoot in or watering with a CCC solution of 125 mg/l) gave less than 0.1 ppm residual CCC even in fruit from the first crop; The fruits from the plants treated with 250 mg/l solution contained 2-3.5 times higher level of CCC residues than fruits from plants which were treated with 125 mg/l CCC solution (Tables 3, 5).

Comparision of the amount of residual CCC in plants from variant (1) (10 mg CCC/plant) and variant (3) (2.4 mg CCC/plant) in the experiment from 1978 (Table 5) shows that plants from variant (1) contained only 33% more residual CCC than plants from variant (3), even though they theoretically received 4 times more CCC. This shows that mainly the CCC applied directly to the plant, and not that added to the soil, has a decisive effect on the size of the residue and retardation effect. CCC added to the soil is quickly degraded (Fig. 1). The studies conducted in 1976 also show that the radioactivity of fruit, leaves, stems and roots of plants from variants (1) and (3) (both treated with a solution of 250 mg CCC/l) was almost identical (the plants differed in the amount of soil left near the roots at the time of transplantation). This also shows that CCC is quickly degraded in the soil. The radioactivity of the roots is at least twice as high (Table 3) as the radioactivity of the leaves, stems or fruits (in the experiment from 1976); in 1978 the radioactivity of the roots was found to be as much as 7 times higher than that of the stems and up to 14 times higher than in leaves (Table 4). In plants which had

Table 3

Radioactivity in fruit, leaves, stems and roots measured by a Geiger-Müller counter (cpm/g dry weight) and residual CCC in fruit (mg/ kg fresh fruit) – measured in a liquid scintillation counter in individual objects and crops. Averages of 6 plants

Experiment: 1976

Treatment	Fruit from consecutive crops								Plants after harvesting cpm/g		
	29.VII		5.VIII		13.VIII		21.VIII				
	cpm/g	mg/kg	cpm/g	mg/kg	counts min/g	mg/kg	cpm/g	mg/kg	leaves	stems	roots
(1) 250 mg CCC/1 ~ 10 mg CCC/plant	18600	0.185	10600	0.122	10600	0.087	4200	0.071	9400	16000	40000
(2) 125 mg CCC/1 ~ 5 mg CCC/plant	11400	0.086	3400	0.042	2000	0.045	0	0.040	11400	7400	21400
(3) 250 mg CCC/1 ~ 10 mg CCC/plant	18600	0.216	11400	0.097	8000	0.116	4000	0.085	10000	18600	43400

Soil from the roots of plants from variants 1 and 2 was removed by gentle shaking before transplanting them, plants from variant 3 were transplanted with the soil still attached.

Table 4

Radioactivity of fruit from individual crops, radioactivity of leaves, stems and roots after vegetation as a function of the dose of CCC used (cpm/g dry weight)

Experiment: 1978

Treatment	Fruit from consecutive crops							Plants after harvesting		
	10.VIII	19.VIII	24.VIII	1.IX	7.IX	19.IX	26.IX	leaves	stems	roots
(1) 250 mg CCC/l* ~10 mg CCC/plant	8888**	6755	5761	3965	3664	895	569	5005	10420	70850
(2) 125 mg CCC/l ~5 mg CCC/plant	2439	1587	1570	1460	959	321	173	4110	5480	28925
(3) 250 mg CCC/l ~2,4 mg CCC/plant	6679	4698	2446	2130	2200	796	423	3467	2619	39602

* – treatment of plants in these variant as in Table 5; ** – radioactivity determined in cpm/g.

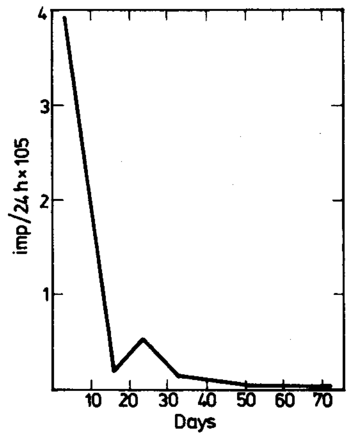

Fig. 1. Degradation of radioactive CCC in compost soil during 2.5 summer months as determined by the amount of liberated $^{14}CO_2$

only been submerged in CCC (variant 3), the roots were also found to be much more radioactivite than the leaves and stems (Table 4). This shows that CCC is accumulated preferentially in the roots, regardless of the manner in which the plant had been treated.

Table 6 shows what per cent of the CCC with which the plant had been treated is found in the fruit. The amount is rather small and amounts to 1.1-6.0‰, depending on the mode of treatment.

Table 5

Residual of CCC (mg/kg fresh mass) determined ZR-16 counter and liquid scintillation counter

Experiment: 1978

Crop	Treatment					
	~10 mg CCC/plant[1]		~5 mg CCC/plant[2]		~2.4 mg CCC/plant[3]	
	ZR-16	Liq. scin.	ZR-16	Liq. scin.	ZR-16	Liq. scin.
10.VIII	0.29	0.35	0.08	0.09	0.22	0.26
19.VIII	0.25	0.28	0.06	0.08	0.16	0.18
24.VIII	0.26	0.22	0.06	0.07	0.08	0.11
1.IX	0.19	0.18	0.05	0.08	0.08	0.11
7.IX	0.14	0.11	0.04	0.05	0.10	0.10
19.IX	0.06	0.07	0.01	0.03	0.03	0.04
26.IX	0.03	0.03	0.01	0.02	0.03	0.03
Average	0.17	0.18	0.04	0.06	0.10	0.12

[1] – the shoot was submerged in a solution of CCC at a concentration of 250 mg CCC/l and the plant watered with the solution (a total of 20 ml solution was used); [2] – as 1, the CCC concentration was 125 mg/l; [3] – only the shoot was submerged in a solution containing 250 mg CCC/l.

Table 6

Radioactivity of fruits from consecutive crops compared to radioactivity applied to the plant (%)

Date of croping	Treatment 10 mg CCC/plant[1]	5 mg CCC/plant[2]	2.4 mg CCC/plant[3]
10.VIII	0.54	0.32	2.72
19.VIII	0.27	0.16	0.70
24.VIII	0.13	0.21	1.24
1.IX	0.56	0.25	0.74
7.IX	0.21	0.08	0.30
19.IX	0.11	0.08	0.21
26.IX	0.02	0.04	0.10
Total	1.83	1.14	6.01

Treatment of plants in each variant as described in Table 5.

DISCUSSION

The results of studies on the effect of CCC on the height of plants confirm the results of other authors (W i t t w e r and T o l b e r t, 1960; K n a v e l, 1969; L e d o v s k i i, 1977, and others) even though a much smaller amount of CCC was used in our experiments.

These studies have shown that after submerging the shoots in a solution of 125 mg/l CCC (dose of 5 mg CCC/plant) as is suggested by B o r k o w s k i (1976) and B o r k o w s k i and J a g ǫ d a (1977) the residual amount of CCC in the tomatoes from the first crop is less than 0.1 mg/kg of fresh fruit. In the following crops, the amount of residual CCC decreased, reaching a level of 0.02 mg/kg fresh fruit in the 7-th crop. M ü l l e r (1972) has also shown that most of the residual CCC is in the fruits of the first crop. Decrease of CCC residues in consecutive crops may result from dilution of CCC in the increasing plant body, or by transport of the substance to the roots and by rinsing it away from leaves by rainfall. This last reason has been pointed to by B o h r i n g (1972).

As in M ü l l e r's study (1972) we also see a correlation between the amount of CCC remaining in the fruit and the amount of CCC with which the plant had been treated.

Accumulation of CCC in the roots of tomato plants is in agreement with D e k h u i j z e n's (1973) observation of the same event in potato plants. This does not happen in all plants; there has been found no tendency to acumulate CCC in wheat roots (B l i n n, 1967; B i r e c k a, 1967).

Studies on the prolonged toxic effects of CCC carried out by N i e p o ł o m s k i et al. (1970) and K o ł o d z i e j c z y k (1972) have shown that the maximum daily dose of CCC well tolerated by rats and which does not cause observable changes in their organisms is 6 mg/kg/day. On this basis, Kołodziejczyk has set the allowed daily dose for humans (using a safety factor of 100) at 0.06 mg/kg/day. Assuming an average weight for humans of 60 kg, taking into account the allowed daily dose of 0.06 mg/kg/day and the amount of residual CCC in fruits from plants treated as suggested for practical purposes, as 0.1 mg/kg, it can be calculated, that the limit of safe consumption for adults is 36 kg (!) of tomatoes daily, for children (assuming a body weight of 10 kg) – 6 kg daily. It should be added, that Niepołomski et al. (1970) and Romanowski (1972b) have shown that CCC does not accumulate in animals, and that tomatoes contain large amounts of choline (a specific antidote for CCC), which significantly lessens the toxic effects of CCC (Ottel, 1966; Zalewski, 1968).

CCC added to compost soil (which contains large amounts of bacteria) is rapidly degraded (Fig. 1). This is in agreement with results obtained by Bohring (1965), also Lisner et al. (1965) have reported that 99% CCC is degraded in 40 days in compost soil, but only 28% of the radioactivity is found in $^{14}CO_2$. Romanowski (1972a) also shows that CCC is degraded during a few days to an unidentified compound with an Rf of 0.64, but the type of soil plays an important role here.

Presoly (1969) reports that plants growing on high peat show a larger retardant effect when treated with CCC and contain more of it than plants growing on compost soil. He explains this by quicker degradation of CCC in compost soil.

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Pozostałości chlormekwatu (CCC) w owocach i innych częściach rośliny pomidorów po potraktowaniu rozsady ^{14}C-CCC

Streszczenie

W dwuletnich badaniach nad stosowaniem CCC w stężeniu 125 mg/l celem zahamowania nadmiernego wzrostu rozsady pomidorów badano pozostałości tego związku w roślinie. Przy dwukrotnym stosowaniu CCC na rozsadę, pozostałości tego związku w owocach z pierwszego zbioru wynosiły 0,09 mg/kg świeżej masy. Ilość pozostałości była tym mniejsza im późniejszy zbiór. W owocach z ostatniego zbioru było 0,02 mg CCC/kg świeżej masy. Zawartość CCC w resztkach liści i łodyg na końcu wegetacji była podobna jak w owocach pierwszego zbioru, natomiast zawartość CCC w resztkach korzeni była kilka razy wyższa niż w owocach. CCC dodany do ziemi kompostowej ulegał szybkiemu rozkładowi.