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Received for review February 6, 1984. Revised manuscript received May 31, 1984. Accepted July 10, 1984. This work has been supported by BMFT and in part by DFG and the Fonds der Chemischen Industrie.

Effects of Pesticide Treatments on the Carotenoid Pigments of Lettuce

Jean Rouchaud,* Chantal Moons, and Joseph A. Meyer

Plots of lettuce were grown in the field in soils treated with one of the herbicides propyzamide or chlorpropham or with their mixture; other plots of lettuce were treated with one of the fungicides benomyl, iprodione, or vinclozolin, and four harvests were made. The treated lettuces, or the ones grown in treated soils, were compared to the untreated lettuces grown in untreated soils. The concentration of total carotene was higher in the lettuces treated with one of the herbicides propyzamide or chlorpropham or with their mixture or with the fungicide iprodione; it was generally the same for the lettuces treated with one of the fungicides benomyl or vinclozolin. Each of the β-cryptoxanthin, lutein, violaxanthin, and neoxanthin contents was increased by each of the propyzamide, chlorpropham, propyzamide plus chlorpropham, and iprodione treatments but not by the benomyl and vinclozolin treatments.

Selected urea herbicides and organophosphoro insecticides incorporated into the soil can either increase or decrease the carotene contents of the carrots grown in these soils (Rouchaud et al., 1982, 1983). The herbicide metachlor decreases the carotene content of sorghum (Wilkinson, 1981). The growth regulator ethephon enhances the β-carotene and the lycopene content of tomato (Buescher and Doherty, 1978). Hormone treatment enhances carotenoid accumulation in wheat leaf (Duysen and Freeman, 1976).

At our knowledge, very few studies have been made about the influence of the pesticide treatments on the provitamin A content of the lettuce. In the present work, we studied the influence of two herbicides applied separately or as a mixture. These are propyzamide, i.e., 3,5-dichloro-*N*-(1,1-dimethylpropynyl)benzamide, and chlorpropham, i.e., isopropyl 3-chlorophenylcarbamate. Three

Laboratory of Phytopathology, Université Catholique de Louvain, 3, Place Croix du Sud, SCI. 15D, 1348 Louvain-la-Neuve, Belgium.

0021-8561/84/1432-1241$01.50/0

fungicides were also assayed separately. These were benomyl, i.e., methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate, iprodione, i.e., 3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide, and vinclozolin, i.e., 3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione. We studied the influence of these pesticides on the carotenoid contents of the lettuce.

EXPERIMENTAL SECTION

Culture and Treatment of the Lettuces. The lettuce culture was made at the Research Station for Vegetables, St-Katelijne-Waver, Belgium. The lettuces (Reskia variety) were sown in a greenhouse on Feb 16, 1983, by using seeds from Nickerson-Zwaan, The Netherlands, and they were planted in the field when they had about six leaves on March 14, 1983, the space between the lettuces being 30 × 30 cm. The plots here called treated and untreated all were of the same soil that, in the past, had received the common and usual fertilization treatments made at equal rates and that have been described (Rouchaud et al., 1982). All experiments were arranged in a randomized block design; there were plots of control (untreated soil and lettuces) and of each treatment. There were four plots (four replications) for each of the control and the treatments. The size of each plot was 1.3 × 10.0 m.

Only one pesticide was applied to each plot, except for the plots that were treated with the sole mixture of propyzamide plus chlorpropham.

For the herbicide-treated soil, an overall surface spray with an aqueous emulsion of the herbicide was made on the finely granulated soil, just before planting of the lettuces; the soil then was raked so that the pesticide was incorporated at a depth of about 6 cm. The treatment was made with one of the following herbicides or with a mixture of them at the recommended rate; Kerb 50 (formulation containing 50 g % of propyzamide) was used at the dose of 12 g of Kerb 50/are; CIPC (formulation containing 400 g of chlorpropham/L) was used at the dose of 15 mL of CIPC/are; a mixture of CIPC and Kerb 50 was used at the dose of 7.5 mL of CIPC plus 6 g of Kerb 50/are.

For the fungicide-treated lettuces, only one treatment was made on May 6, 1983, by spraying the lettuces with an aqueous emulsion of the fungicide when the lettuces had about 12 leaves. The treatment was made with one of the following fungicides at the recommended rate; Benlate (formulation containing 50 g % of benomyl) was used at the dose of 15 g of Benlate/are; Rovral (formulation containing 50 g % of iprodione) was used at the dose of 15 g of Rovral/are; Ronilan (formulation containing 50 g % of vinclozolin) was used at the dose of 10 g of Ronilan/are.

Four harvests were made, the last harvest being made 24 days after the first one. The harvested lettuces from each of the plots were healthy, no fungi attack being observed. For the plots not treated with an herbicide, the weeds were taken off by hand so that there were no weeds in any of the plots during culture.

Sampling and Extraction of the Lettuces. For analysis, one sample was made from each plot in the following way. Twenty lettuces were collected at random from each plot, stored at 4 °C for 2 days before analysis, washed in running water, and dried by blotting with paper towels. From each lettuce a spherical sector was cut off that corresponded to one fourth of the lettuce. The sectors were mixed and diced, the small pieces were mixed, 15 g of the mixture was taken for dry weight measurement, and 100 g was taken for carotenoid analysis.

The sample of 100 g was extracted with 300 mL of acetone in a Sorvall omnimixer at 8000 rpm during 5 min, the mixture was filtered, the extraction of the solids was repeated 2 times with 2 × 300 mL of acetone, and the filtrates were gathered and mixed. The volume was concentrated to about 50 mL by evaporation under vacuum at 40 °C in a rotavapor, as it was for all the concentrations by evaporation mentioned hereafter. Fifty milliliters of 1-butanol, 100 mL of methanol, and 3 mL of 40 g % of KOH in methanol were added, the mixture was heated at 55 °C for 20 min, 100 mL of a 10 g % solution of Na_2SO_4 in water was added, the volume was reduced to about 120 mL by evaporation, and the mixture was extracted 3 times by 3 × 150 mL of the mixture hexane–acetone–absolute ethanol–toluene (40:7:6:7). The organic layers were gathered and evaporated to dryness, and the residue was dissolved in 20 mL of acetone, giving solution A.

Identification and Measurement of the Carotenoids. The visible spectra (vis) were recorded with a Beckmann Acta CIII UV–visible spectrophotometer (λ_{max} in nm). The infrared (IR) spectra were obtained with a Perkin-Elmer 297 spectrometer; ($\bar{\nu}_{max}$ in cm^{-1}). The H NMR spectra were obtained in $CDCl_3$ with a Bruker Cryospec WM 250, i.e., 250-MHz spectrometer (δ). Mass spectrometry (MS) was carried out on a Varian-MAT 311 at 70 eV; (mass numbers in m/e).

Column chromatography of the pigments on a mixture of 1:1 (w/w) activated magnesia and Hyflo Super-Cel (Horwitz, 1975) using mixtures of hexane and acetone as eluants was not convenient. Acetone was transformed on the column into aldol condensation products; this was shown by elution of the pure hexane–acetone solvent. Moreover, the pigments also were decomposed on the column; this was shown by quantitative visible absorption spectrometry and, more clearly, by the H NMR spectra of the column chromatography fractions. Changing the ratio magnesia/Hyflo Super-Cel or the nature of the elution solvents was inefficient to avoid pigment decomposition. For these reasons, that chromatography procedure was abandoned and the silica gel and alumina thin-layer chromatography (TLC) procedures were assayed.

Thin-layer chromatography (TLC) procedures were used that do not decompose the carotenoids into unidentified products. This was shown by the separate TLC chromatography of standards of each of the *all-trans*-β-carotene and the xanthophylls. Each sample was analyzed by two different TLC procedures. The first TLC procedure used silica gel plates and the second one aluminum oxide plates. The aluminum oxide separated the β-carotene stereoisomers and the xanthophylls of the lettuce (β-cryptoxanthin, lutein, violaxanthin, and neoxanthin) without transforming them at all; the same was observed with the silica gel plates except that the violaxanthin and the neoxanthin of the lettuce were transformed respectively into auroxanthin and luteoxanthin on the silica gel plates.

In the first TLC procedure, 5 mL of solution A was concentrated and applied as a band on a Merck TLC plastic sheet of silica gel 60 F254, 20 × 20 cm, layer thickness 0.2 mm, eluted with hexane–$CHCl_3$, 90:10, giving a band of R_f = 0.90 containing the carotenes and a band of R_f = 0.00 containing the xanthophylls. This last was scraped off, extracted with acetone, TLC chromatographed again on a silica gel plate, and eluted with $CHCl_3$–acetone, 80:20, giving four bands, R_f: β-cryptoxanthin, 0.82; lutein, 0.61; auroxanthin, 0.39; luteoxanthin, 0.18.

In the second TLC procedure, 5 mL of solution A was concentrated, TLC chromatographed on a Merck aluminum oxide 60 F254 (type E), 20 × 20 cm, layer thickness 0.25 mm, and eluted with hexane–$CHCl_3$ 90–10, giving a band of R_f = 0.95 containing the carotenes and a band of

R_f = 0.00 containing the xanthophylls. This last was scraped off, extracted with acetone, TLC chromatographed again on an aluminum oxide plate, and eluted with $CHCl_3$–acetone, 60:40, giving four bands, R_f: β-cryptoxanthin, 0.84; lutein, 0.59; violaxanthin, 0.35; neoxanthin, 0.14.

The carotene TLC band contained the mixture of the β-carotene stereoisomers, which is called here the total carotene (Table II). That band was scraped off, extracted with hexane, and, for routine analyses, measured by visible absorption spectrometry at 436 nm using 196 g L^{-1} cm^{-1} as the absorption coefficient (Horwitz, 1975).

The distribution of the isomers in the mixture of β-carotene stereoisomers was measured by TLC on aluminum oxide by using the same plate as above and hexane as the eluant; the order of elution of the isomers was the same as that observed with the column chromatography on a 1:6 (w/w) mixture of magnesium hydroxide and calcium hydroxide using 1.5% *p*-methylanisole in petroleum ether as the eluant (Sweeney and Marsh, 1970). The TLC band corresponding to *all-trans*-β-carotene was extracted; these extracts from several lettuce analyses were gathered, and the *all-trans*-β-carotene isolated in that way was further analyzed by IR, H NMR, and MS. *all-trans*-β-Carotene: vis (EtOH) 427 nm, 449, 475; vis (acetone) 423 nm, 452, 478. IR (KBr) 3050–2800, 1625, 1560, 970, 830 cm^{-1}; H NMR δ 1.03 (s, CH_3 16, 17, 16′, 17′), 1.72 (s, CH_3 18, 18′), 1.98 (s, CH_3 19, 19′, 20, 20′), 1.2–2.0 (m, CH_2), 6.0–6.8 (m, vinyl protons); MS *m/e* 536 (M), 457 (M − 79), 444 (M − 92), 430 (M − 106), 399 (M − 137), 378 (M − 158).

The chromatographic bands of the pure xanthophylls were scraped off, extracted with acetone, and measured by UV-visible spectrometry using the extinction coefficients given in the literature for each of these compounds at the maximal absorbance. The extracts, from several lettuce samples, corresponding to the same TLC band were gathered, and the pure products were further analyzed by IR, H NMR, and MS. β-Cryptoxanthin: vis (EtOH) 428 nm, 449, 473; vis (acetone) 428 nm, 449, 474; IR (KBr) 3300–3600, 2950, 2925, 1550, 990, 950 cm^{-1}; H NMR δ 1.03 (s, CH_3 16′, 17′), 1.07 (s, CH_3 16, 17), 1.72 (s), CH_3 18, 18′), 1.73 (s, CH_3 18, 18′), 1.97 (s, CH_3 19, 20, 19′, 20′), 1.2–2.0 (m, CH_2), 4.00 [s, H-C(3)], 5.20–5.45 [m, H-C(7)], 6.0–6.8 (m, vinyl protons); MS *m/e* 552 (M), 534 (M − H_2O), 460 (M − 92), 446 (M − 106). Lutein: vis (EtOH) 422 nm, 445, 474; vis (acetone) 420 nm, 443, 470; IR ($CHCl_3$) 3615, 3460, 2960, 2920, 2860, 1600, 1572, 967 cm^{-1}; H NMR δ 0.85 (s, CH_3 16′, 17′), 0.99 (s, CH_3 16′, 17′), 1.08 (s, CH_3 16), 1.26 (s, CH_3 17), 1.63 (m, CH_3 18′), 1.74 (s, CH_3 18), 1.97 (s, CH_3 19, 20, 20′), 1.91 (s, CH_3 19′), 1.2–2.0 (CH_2), 2.2–2.4 [H-C(6′)], 3.75 [s, H-C(3)], 4.15–4.28 [H-C(3′)], 5.25–5.45 [m, H-C(7)], 5.53 [m, H-C(7′)], 6.0–6.8 [m, vinyl protons]; MS *m/e* 568 (M), 550 (M − H_2O), 532 (M − $2H_2O$), 476 (M − 92), 462 (M − 106), 458 (476 − H_2O), 444 (462 − H_2O). Violaxanthin: vis (EtOH) 417 nm, 440, 469; vis (acetone) 416 nm, 440, 470; IR (KBr) 3300–3600, 3020, 2950, 2850, 1550, 980, 950, 780 cm^{-1}; H NMR δ 0.95 (s, CH_3 16, 16′), 1.12 (s, CH_3 17, 17′), 1.16 (s, CH_3 18, 18′), 1.17 (s, CH_3 18, 18′), 1.89 (s, CH_3 19, 19′), 1.92 (s, CH_3 20, 20′), 1.2–2.0 (m, CH_2), 3.75 [s, H-C(3)], 3.85 [s, H-C(3)], 4.20–4.30 [H-C(3′)], 5.2–5.5 [m, H-C(7, 7′)], 6.0–6.8 (m, vinyl protons); MS *m/e* 600 (M), 584 (M − 16), 582 (M − H_2O), 564 (M − $2H_2O$), 520 (M − 80), 508 (M − 92), 494 (M − 106). Luteoxanthin: vis (EtOH) 396 nm, 420, 446; vis (acetone) 398 nm, 422, 448; IR (KBr) 3300–3600, 3020, 2950, 2850, 1550, 980, 950, 780 cm^{-1}; H NMR δ 0.85 (s, CH_3 16), 0.88 (s, CH_3 16), 1.07 (s, CH_3 16′), 1.10 (s, CH_3 16′), 1.18 (m, CH_3 18), 1.26 (s, CH_3 17), 1.34 (s, CH_3 17′), 1.62 (s, CH_3 20, 20′), 1.68 (m, CH_3 18′), 1.80 (s, CH_3 19), 1.96 (s, CH_3 19′), 1.2–2.0 (m, CH_2), 2.63 [s, H-C(8′)], 3.75 [s, H-C(3)], 3.78 [s, H-C(3)], 4.15–4.35 [H-C(3′)], 5.10 [s, H-C(7)], 5.20 [s, H-C(7)], 5.26 [s, H-C(7′)], 5.32 [s, H-C(7′)], 6.0–6.8 (m, vinyl protons); MS *m/e* 600 (M), 584 (M − 16), 582 (M − H_2O), 564 (M − $2H_2O$), 520 (M − 80), 508 (M − 92), 494 (M − 106). Auroxanthin: vis (EtOH) 381 nm, 402, 427; vis (acetone) 380 nm, 401, 427; IR (KBr) 3300–3600, 3020, 2950, 2850, 1550, 980, 950, 780 cm^{-1}; H NMR δ 0.87 (s, CH_3 16′), 0.88 (s, CH_3 16′), 1.17 (s, CH_3 16), 1.20 (s, CH_3 16), 1.26 (s, CH_3 17′), 1.33 (s, CH_3 17), 1.62 (s, CH_3 18′), 1.68 (s, CH_3 18), 1.71 (s, CH_3 19′), 1.81 (s, CH_3 19), 1.94 (s, CH_3 20, 20′), 1.2–2.0 (m, CH_2), 2.64 [s, H-C(8, 8′)], 3.75 [s, H-C(3)], 3.78 [s, H-C(3)], 4.17–4.28 [H-C(3′)], 5.07 [s, H-C(7, 7′)], 5.17 5.25 [s, H-C(7, 7′)], 5.26 [s, H-C(7, 7′)], 6.0–6.8 (m, vinyl protons); MS *m/e* 600 (M), 584 (M − 16), 582 (M − H_2O), 564 (M − $2H_2O$), 520 (M − 80), 508 (M − 92), 494 (M − 106). Neoxanthin: vis (EtOH) 415 nm, 438, 467; vis (acetone) 417 nm, 440, 468; IR ($CHCl_3$) 3597, 1923, 965 cm^{-1}; H NMR δ 0.96 (s, CH_3 16′, 17′), 1.14 (s, CH_3 16′, 17′), 1.17 (s, CH_3 18′), 1.33 (s, CH_3 16, 17), 1.06 (s, CH_3 18), 1.79 (s, CH_3 19), 1.91 (s, CH_3 19′), 1.94 (s, CH_3 20, 20′), 1.2–2.0 (m, CH_2), 3.76 [s, H-C(3′)], 3.87 [s, H-C(3′)], 4.20–4.45 [m, H-C(3, 5)], 5.1–5.4 [m, H-C(7′, 8)], 6.0–6.8 (m, vinyl protons); MS *m/e* 600 (M), 582 (M − H_2O), 564 (M − $2H_2O$), 520 (M − 80), 508 (M − 92), 502 (M − H_2O − 80), 490 (M − H_2O − 92), 484 (M − $2H_2O$ − 80).

Moreover, the epoxides were detected by their characteristic visible hypsochromic spectral shift in the presence of a solution of traces of hydrochloric acid in methanol: violaxanthin was transformed into auroxanthin, and neoxanthin was transformed successively into luteoxanthin and auroxanthin; after some time, or more rapidly with higher concentrations of hydrochloric acid, all these solutions became blue-green (Karrer, 1948).

For the statistical analysis of the results, differences between means were tested for significance by application of the T method (method of Tukey) (Scheffé, 1959).

RESULTS AND DISCUSSION

The degree of heading of the lettuce was appreciated visually by using a scale from 1 to 10, 10 corresponding to strongly headed lettuces. The degree of heading was related to the mean unitary fresh weight of the lettuce (mean unitary fresh weight, g/mean degree of heading): 200/2; 300/5; 400/7–8; 500/9; 600/10.

The degree of darkness of the green color of the leaves of the lettuces was appreciated visually by using a scale from 1 to 10, 10 corresponding to dark green blades. At each of the four harvests, the green color of the external leaves was always about 8.0. The green color of the internal leaves was related to the mean unitary fresh weight of the lettuce (mean unitary fresh weight, g/mean degree of darkness of the green color): 150/6.0; 200/5.0; 300/5.0; 400/4.5; 500/3.5; 600/3.0.

The mean percentage of dry matter of the lettuce decreased progressively with the increase of the mean unitary fresh weight of the lettuce. For the lettuces whose unitary weights were lower than 300 g, the mean percentage of dry matter was about 8.5. For the lettuces whose unitary fresh weights were higher than 300 g, the mean percentage of dry matter was 6.4.

The degrees of heading and of darkness of the green color, and the percentage of dry matter of the lettuces of the same weights did not vary significantly according to the pesticide treatment.

Table I gives the mean unitary fresh weights of the lettuces at each harvest. Relative to the control (lettuces

Table I. Unitary Mean Fresh Weights of the Lettuces

pesticide treatment	unitary fresh weights of the lettuces, g,[d] for harvest date[e]			
	5/20	5/26	6/1	6/7
control	191 ± 16	340 ± 14	483 ± 20	626 ± 22
propyzamide	191 ± 15[c]	291 ± 12[b]	453 ± 20[c]	508 ± 22[a]
chlorpropham	171 ± 14[c]	302 ± 13[c]	391 ± 16[a]	487 ± 18[a]
propyzamide plus chlorpropham	265 ± 23[a]	400 ± 16[a]	542 ± 18[a]	687 ± 32[a]
benomyl	207 ± 19[c]	308 ± 14[c]	420 ± 17[a]	539 ± 18[a]
iprodione	307 ± 26[a]	421 ± 23[a]	583 ± 25[a]	716 ± 25[a]
vinclozolin	281 ± 18[a]	379 ± 16[c]	547 ± 21[a]	658 ± 27[c]

[a-c] Significantly different from the control at the 1% (*a*) and 5% (*b*) level or nonsignificant (*c*), respectively. [d] Means ± SD of the weights of 20 lettuces. [e] Month and day, year 1983.

not treated by fungicide and grown in a soil not treated by herbicide), the unitary fresh weights of the lettuces were higher for the lettuces treated with the herbicide mixture propyzamide plus chlorpropham or with the fungicide iprodione; the other pesticide treatments had generally no effect on the unitary fresh weights.

Standards of the *all-trans*-β-carotene and of each of the xanthophylls were separately submitted to the hydrolysis and the TLC procedures; their measurements and their identifications indicated recoveries of 84–100%, and none of the compounds was decomposed, except for the mentioned transformations on the silica gel TLC plates.

The distribution of the carotene stereoisomers did not change significantly with the harvest date and the pesticide treatment. The mean distribution was: neo-β-carotene B, 5%; *all-trans*-β-carotene, 77%; neo-β-carotene U, 18%. The biological value of this mixture was 86.5%/mol, based on biopotency values relative to *all-trans*-β-carotene as 100%/mol (Zechmeister, 1962). The changes observed for the total carotene concentration with the pesticide treatment thus completely corresponded to changes of the provitamin A value of the lettuces.

Table II gives, for each harvest, the concentration of total carotene, i.e., the concentration of the sum made up of each of the β-carotene stereoisomers.

For the total carotene content per fresh weight (f.w.) in lettuce, McCance and Widdowson (1960) report 1000 μg/100 g of f.w., and Watt and Merrill (1966) report 580 μg/100 g. However, the total carotene content depends on several factors. According to the level of NPK fertilization, the extreme values observed were 1020 and 4920 μg/100 g of f.w. (Scharrer and Bürke, 1953). According to the light intensity during culture, and thus to the period of the year during which the culture was made, the extreme values observed were 500 and 1600 μg/100 g of f.w. (Rinno and Becker, 1965; Hulewicz and Kalbarczyk, 1976).

This work shows (Table II) that the total carotene content also decreased when the weight of the lettuces increased during their development; the lettuces harvested at 1–6 had their normal weight for harvest in commercial horticulture, i.e., 400–500 g. Moreover, the total carotene content was influenced by the pesticide treatment. Relative to the control lettuces, the concentration of total carotene was higher in the lettuces treated with one of the herbicides propyzamide or chlorpropham or with their mixture or with the fungicide iprodione; it was generally the same for the lettuces treated with one of the fungicides benomyl or vinclozolin.

Comparison of lettuces of similar fresh weights indicates that when the lettuces were not heavier than 300 g they had similar concentrations of total carotene, independently of the pesticide treatment. When they were heavier, the lettuces treated with the herbicide mixture propyzamide plus chlorpropham or with the fungicide iprodione had higher carotene concentration than the control lettuces; the lettuces treated with the herbicide chlorpropham or with the fungicide benomyl had generally lower total carotene concentrations; the lettuces treated with the herbicide propyzamide or with the fungicide vinclozolin had similar total carotene concentrations. The effect of

Table II. Carotenoid Content of the Fresh Lettuce

pesticide treatment	harvest date, month and day (year 1983)	carotenoid content, μg/100 g of fresh lettuce[d]				
		total carotene	β-cryptoxanthin	lutein	violaxanthin	neoxanthin
control	5/20	2523 ± 111	172 ± 10	2573 ± 121	1111 ± 49	433 ± 21
	5/26	2308 ± 97	160 ± 9	2185 ± 92	1188 ± 52	460 ± 23
	6/1	1585 ± 70	112 ± 7	1553 ± 68	750 ± 32	375 ± 20
	6/7	755 ± 35	60 ± 4	856 ± 40	392 ± 18	202 ± 11
propyzamide	5/20	2913 ± 131[a]	198 ± 12[a]	2924 ± 123[a]	1312 ± 56[a]	498 ± 26[a]
	5/26	2648 ± 117[a]	189 ± 11[a]	2579 ± 113[a]	1406 ● 66[a]	537 ± 27[a]
	6/1	1826 ± 77[a]	134 ± 8[a]	1900 ± 89[a]	918 ± 40[a]	452 ± 24[a]
	6/7	914 ± 40[a]	74 ± 5[a]	1051 ± 47[a]	468 ± 20[a]	264 ± 13[a]
chlorpropham	5/20	2959 ± 139[a]	191 ± 12[c]	3015 ± 127[a]	1279 ± 55[a]	490 ± 25[a]
	5/26	2707 ± 119[a]	189 ± 11[a]	2608 ± 115[a]	1384 ± 61[a]	543 ± 29[a]
	6/1	1863 ± 78[a]	137 ± 8[a]	1910 ± 86[a]	919 ± 43[a]	449 ± 22[a]
	6/7	964 ± 42[a]	70 ± 4[a]	1063 ± 50[a]	503 ± 21[a]	236 ± 13[a]
propyzamide plus chlorpropham	5/20	2890 ± 130[a]	186 ± 12[c]	3015 ± 142[a]	1335 ± 56[a]	482 ± 24[a]
	5/26	2737 ± 120[a]	197 ± 12[a]	2708 ± 119[a]	1446 ± 68[a]	560 ± 27[a]
	6/1	1845 ± 77[a]	130 ± 8[a]	1872 ± 79[a]	901 ± 40[a]	445 ± 24[a]
	6/7	1018 ± 48[a]	69 ± 4[a]	1122 ± 49[a]	515 ± 22[a]	257 ± 14[a]
benomyl	5/20	2544 ± 114[c]	171 ± 10[c]	2461 ± 108[c]	1149 ± 48[c]	492 ± 24[a]
	5/26	2518 ± 111[b]	157 ± 9[c]	2145 ± 97[c]	1167 ± 51[c]	451 ± 23[c]
	6/1	1492 ± 63[c]	108 ± 7[c]	1510 ± 71[c]	703 ± 33[c]	391 ± 21[c]
	6/7	597 ± 26[a]	62 ± 4[c]	874 ± 37[c]	400 ± 17[c]	208 ± 11[c]
iprodione	5/20	2811 ± 118[a]	190 ± 11[c]	2995 ± 135[a]	1250 ± 59[a]	488 ± 26[a]
	5/26	2612 ± 110[a]	189 ± 11[a]	2585 ± 109[a]	1409 ± 59[a]	549 ± 26[a]
	6/1	1849 ± 81[a]	135 ± 9[a]	1819 ± 85[a]	925 ± 40[a]	441 ± 23[a]
	6/7	1023 ± 48[a]	75 ± 5[a]	1099 ± 48[a]	523 ± 23[a]	236 ± 12[a]
vinclozolin	5/20	2518 ± 118[c]	170 ± 11[c]	2474 ± 111[c]	1103 ± 52[c]	509 ± 25[a]
	5/26	2229 ± 98[c]	162 ± 10[c]	2258 ± 106[c]	1207 ± 53[c]	428 ± 21[c]
	6/1	1469 ± 62[b]	120 ± 7[c]	1561 ± 69[c]	763 ± 33[c]	371 ± 20[c]
	6/7	756 ± 34[c]	65 ± 4[c]	884 ± 37[c]	389 ± 16[c]	225 ± 12[b]

[a-c] As in Table I. [d] Means ± SD of four replications.

the pesticide treatment on the total carotene concentration of the lettuce thus was more apparent during the increase of the weight of the lettuce beyond 300 g, an increase that was joined to the decrease of the total carotene concentration.

Each of the β-cryptoxanthin, lutein, violaxanthin, and neoxanthin contents were increased by each of the propyzamide, chlorpropham, propyzamide plus chlorpropham, and iprodione treatments but not by the benomyl and vinclozolin treatments. The percentages of these increases were similar for each of these xanthophylls and had about the same values as the ones of the corresponding total carotene contents. The distribution of the xanthophylls thus was not changed by the pesticide treatments nor by the harvest date, and it amounted to about the following, %: β-cryptoxanthin, 4; lutein, 56; violaxanthin, 26; neoxanthin, 14.

β-Cryptoxanthin is the sole of the xanthophylls observed here which is provitamin A active. The changes observed here for its contents through the pesticide treatments thus contribute also to the provitamin A value of the lettuce.

The violaxanthin cycle is a deepoxidation sequence in which violaxanthin is converted to zeaxanthin through the intermediate antheraxanthin; the reverse sequence of epoxidation occurs by a mechanism that is different from that of deepoxidation (Sapozhnikov et al., 1957). The violaxanthin cycle is related to photosynthesis. Sapozhnikov (1973) proposed that the epoxide oxygen originates from water, that molecular oxygen serves as an oxidant of water, and that the violaxanthin cycle functions as a pathway for photosynthetic oxygen evolution; with *Chlorella*, indeed, violaxanthin incorporated O^{18} from H_2O^{18} in the dark, and that epoxide O^{18} was lost in the light (Sapozhinikov et al., 1965). Takeguchi and Yamamoto (1968) reported incorporation of ^{18}O also from $^{18}O_2$ by violaxanthin in leaves. The step of the photosynthesis procedures at which the violaxanthin cycle collaborates is thus not known with certainty. The violaxanthin cycle is related to the oxidoreduction of some electron carrier of the photosystems (Siefermann and Yamamoto, 1975). The enzyme that catalyzes violaxanthin deepoxidation has been isolated from lettuce chloroplasts, and its chemical structure has been determined (Yamamoto and Higashi, 1978).

In the present work, the concentrations of antheraxanthin and zeaxanthin in lettuce were very low and were not measured; only the violaxanthin content was measured. The lettuce chloroplasts were not isolated, and their biochemical activities in the light and in the dark were not measured. However, propyzamide, chlorpropham, propyzamide plus chlorpropham, and iprodione treatments increased the violaxanthin content (and those of total carotene and of all the other xanthophylls) of the lettuce; propyzamide plus chlorpropham and iprodione increased the weight of the lettuces. These facts suggest the hypothesis that these pesticide treatments increased the violaxanthin cycle activity and the photosynthesis activity of the lettuces.

ACKNOWLEDGMENT

We are grateful to Dr. F. Benoit, N. Ceustermans, and F. Van Linden (Vegetable Research Station, St-Katelijne-Waver, Belgium), who made the culture and the pesticide treatments of the lettuces.

Registry No. Propyzamide, 23950-58-5; chlorporpham, 101-21-3; benomyl, 17804-35-2; iprodione, 36734-19-7; vinclozolin, 50471-44-8; β-cryptoxanthin, 472-70-8; lutein, 127-40-2; violaxanthin, 126-29-4; luteoxanthin, 1912-50-1; auroxanthin, 27785-15-5; neoxanthin, 27780-09-2; neo-β-carotene B, 6811-73-0; neo-β-carotene U, 13312-52-2; *all-trans*-β-carotene, 7235-40-7.

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Received for review March 12, 1984. Accepted July 9, 1984. This work was supported by Grant 2035A from the Institut pour l' Encouragement de la Recherche Scientifique dans l' Industrie et l' Agriculture (IRSIA, Belgium).