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Effects of Weed Interference and Herbicides on Nitrate and Carotene Accumulation in Lettuce

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Nitrate and carotene accumulation in field-grown lettuce suffering natural weed interference or protected by hoeing or herbicides was examined in a 2-year study. At the time of maturation weed interference had caused a reduction of nitrates in lettuce about equal to that of dry matter and a 2–4-fold reduction of carotenes. Carotene accumulation is therefore highly sensitive to weed interference. Nitrate content was higher in mature lettuce than in lettuce of a less advanced stage. All herbicides used (alachlor, pendimethalin, propyzamide) accelerated final level accumulation of nitrates, probably inducing a physiological state of maturation. Alachlor and pendimethalin, which are not as selective in lettuce as propyzamide, reduced carotenes in the year of heavier rain although they did not reduce the dry weight.

High-quality green vegetables are expected to contain the lowest concentrations of nitrates and to be rich in carotenoids. Concern over nitrates is because of their reduction to nitrites, which are involved in infant methemoglobinemia and in formation of the carcinogenic nitrosamines. Carotenoids (carotenes, xanthophylls), especially carotenes, are essential in our diet, serving as precursors of vitamin A.

The effects of N fertilization and of environmental factors on nitrate accumulation have been extensively studied (Szwonek, 1986). Efforts are currently made to develop cultural methods to reduce nitrates in vegetables (Roorda van Eysinga, 1984). Little is known, however, of the effect that weed interference and weed control measures may have on nitrate accumulation. Nitrate reductase activity has been increased by 2,4-D (Beevers et al., 1963) or simazine (Tweedy and Ries, 1967) under certain conditions. Subtoxic concentrations of herbicides have increased nitrate uptake in some cases (Ries, 1980).

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Table I. Air Temperature and Rainfall in the Location of the Experiments during the 1986 and 1987 Growing Periods

	mean temp,[a] °C					
	minimum		maximum		rainfall, mm	
month	1986	1987	1986	1987	1986	1987
January	4.1	3.8	12.5	12.0	0	55.8
February	4.1	4.9	11.7	10.4	0	70.8
March	5.5	−0.5	12.8	9.8	38.0	73.9
April	7.7	6.2	20.3	17.5	13.4	75.1

[a] Values are the monthly averages of daily minimum or maximum temperatures.

To our knowledge, weed interference effects on carotene content of vegetables have not been studied. On the other hand, it has been reported that some herbicides either increase or decrease carotenes in selected crops (Sweeney and Marsh, 1971; Rouchaud et al., 1983, 1984).

The purpose of this study was to examine how nitrate and carotene content of lettuce is affected by weed interference under normal field conditions. The effects of herbicides used in lettuce were also examined.

MATERIALS AND METHODS

Lettuce Culture and Weed Control. Romaine lettuce of the cultivar Paris Cos was grown in the field during the January–April period in 1986 and 1987. The field was located in the vegetable-producing area of Marathon, near Athens, Greece, and was characterized by a SL soil with 15, 21, and 1.78% of clay, silt, and organic matter content, respectively, and pH 7.3. Important differences in weather between the 2 years are given in Table I.

Lettuce was transplanted in the field in January (on the 19th in 1986 and the 5th in 1987), received a fertilization with 98 kg of N/ha as NH_4NO_3 early in March , and sprinkle-irrigated and sprayed with fungicide as needed.

The following weed control treatments were applied with the purpose of examining their influence on nitrate and carotene content of lettuce: (1) one hoeing in the first week of March, when most of the weeds had emerged and were still in early stages of growth (cotyledons to four true leaves); (2) application of 2.5 kg/ha alachlor to the soil surface 1 or 2 days before transplanting; (3) application of 2 kg/ha pendimethalin, similar to alachlor; (4) application of 2 kg/ha propyzamide 2 or 3 days after transplanting; (5) no control, allowing the weeds to compete with the crop.

In the 1986 experiment, weed control treatments were applied in 2 × 5 m plots, each plot including 8 rows and 20 plants per row. The plots were arranged in a randomized complete block design with four replications. Sampling was made when most plants had headed (14 April). Twenty plants were randomly picked from the middle four rows of each plot.

In the 1987 experiment, treatments were applied in unreplicated 2 × 25 m strips on a preselected uniform patch of the field. A first sampling was made on 31 March, when lettuce was already well-developed but not headed yet, and a second one 10 days later at heading. For each weed control treatment 4 × 20 plants were picked from the middle rows along the entire length of the strip.

The whole plant samples were soon weighed and stored at 4 °C and then subsampled for analysis, in sets corresponding every time to a replication of treatments, within 48 h of harvest. The lettuces of each sample were washed in running water and dried with blotting paper, and then a spherical sector corresponding to a fourth of a lettuce was cut off for each. The 20 sectors were chopped and mixed; a 50-g portion was taken for dry weight and nitrate determination and 100 g for carotenoid analysis.

Nitrate Determination. The samples were dried at 70 °C for 48 h and ground with a Model 448 Casela mill until passing through a 1-mm screen. Ground sample portions (400 mg) were shaken in 50 mL of deionized water and filtered through glass wool.

Nitrates in the filtrate were measured on a Type 152223000 Ingold nitrate ion selective electrode. To eliminate possible bicarbonate and organic anion interferences, 1 mL/sample of a 0.9 M $Al_2(SO_4)_3$ solution was used for ionic strength adjustment (Paul and Carlson, 1968).

Carotenoid Extraction and Determination. Carotenes and xanthophylls were extracted and separated according to the method of the Association of Official Analytical Chemists (1975) as modified by Rouchaud et al. (1984). Work was performed in dim light or darkness.

The sample of 100 g was initially extracted with 3 × 300 mL of acetone and then subjected to hot saponification by heating at 55 °C for 20 min following addition of 1-butanol, methanol, and methanolic KOH. Finally, a solution of Na_2SO_4 was added, and the extract was partitioned three times in a mixture of hexane–acetone–absolute ethanol and toluene. The organic layers were combined and evaporated to dryness. The residue was redissolved in 10 mL of acetone, and 2.5 mL of this solution was reduced in volume and applied as a band on a Merck TLC glass plate of silica gel 60, 20 × 20 cm, layer thickness 0.25 mm. The plate was developed with hexane–chloroform (90:10), giving a band of R_f 0.90 containing the carotenes and a band of R_f 0.00 containing the xanthophylls. The former band was scraped off, extracted with *n*-hexane, and for routine analysis measured spectrophotometrically. The xanthophyll band was also scraped off, eluted with acetone, and measured.

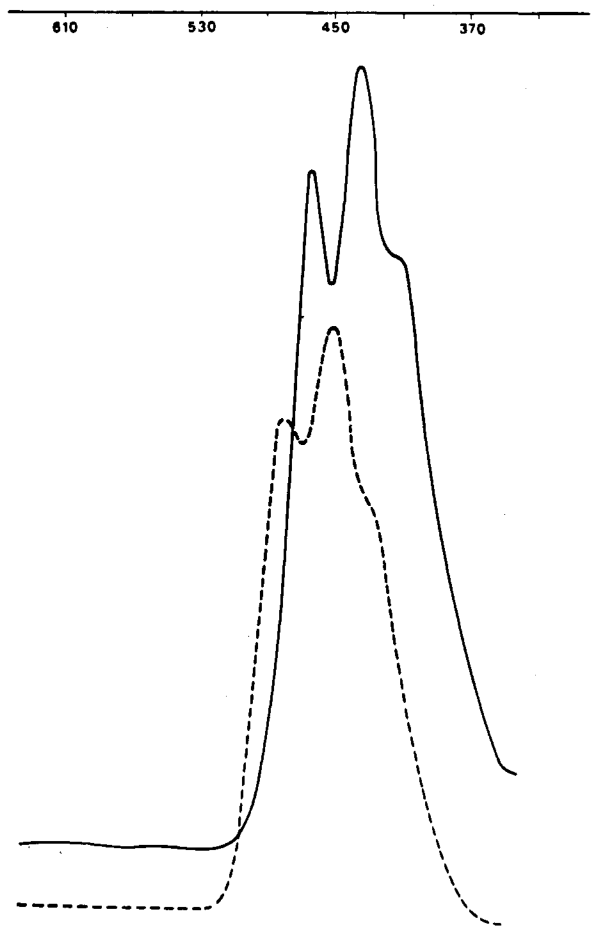

Figure 1. Visible absorption spectra of lettuce xanthophyll (—) and carotene (- - -) solutions, in acetone and *n*-hexane, respectively, after the TLC separation step of the procedure.

Spectrophotometric determination of carotenes and xanthophylls was performed with an SP8-200 UV/vis Pye Unicam spectrophotometer. Absorption spectra obtained for carotenes and xanthophylls (Figure 1) verify reliability of methodology as they are in full agreement with those described in the literature. Carotenes were quantitatively determined by reading absorbance at 451 nm and using Sigma C 9750 crystalline *trans*-β-carotene as a standard. Quantitative differences among treatments in their total xanthophyll content were judged from differences in eluate absorbance at 445 nm.

To examine possible qualitative differences among treatments in composition of carotenes and xanthophylls, combined eluates (from four replications in 1987) were further analyzed by TLC. Carotene components were separated by TLC on the above silica gel with *n*-hexane–toluene (70:30) as the eluant and on Merck aluminum oxide 60 glass plates (20 × 20 cm, 0.25-mm layer thickness) developed with *n*-hexane. Xanthophylls were separated

Table II. Fresh Weight (g/Lettuce) and Dry Weight (%) of Lettuces at the Time of Sampling (Each Value Is the Mean of 20 Lettuces)[a]

weed control treatment	1986 14 April fresh weight	1986 14 April dry weight	1987 31 March fresh weight	1987 31 March dry weight	1987 10 April fresh weight	1987 10 April dry weight
none	308*	5.9*	180*	4.8*	240*	4.1*
hoeing	558	7.7	275	5.8	455	5.4
alachlor	468	7.7	260	5.9	537*	5.4
pendimethalin	519	7.9	260	6.1	407	5.5
propyzamide	701*	7.4	265	5.8	492	5.2

[a] Asterisks indicate values statistically different from respective control (hoeing) value at $P = 0.05$.

Table III. Nitrate Content of Field-Grown Lettuce during 1986 and 1987[a]

weed control treatment	$\mu g\ NO_3/g$ fresh weight: 1986 14 April[b]	1987 31 March	1987 10 April[b]
none	1398*	563	895*
hoeing	1779	507	1329
alachlor	1667	808*	920*
pendimethalin	1755	1017*	1110
propyzamide	1523*	953*	1249

[a] Asterisks indicate values statistically different from respective control (hoeing) value at $P = 0.05$. [b] Lettuce had reached the stage of heading at this time of harvest.

Table IV. Carotenoid Content of Field-Grown Lettuce Harvested at the Time of Heading[a]

weed control treatment	β-carotene, μg/g fresh wt: 1986	1987	xanthophylls (A445): 1987
none	3.6*	7.3*	0.548*
hoeing	20.8	15.0	0.737
alachlor	21.4	10.2*	0.722
pendimethalin	21.6	11.9*	0.708
propyzamide	20.2	16.2	0.813*

[a] Asterisks indicate values statistically different from respective control (hoeing) value at $P = 0.05$.

by TLC on the aluminum oxide plates with chloroform-acetone (60:40).

Statistical Analysis. All results were statistically analyzed by performing analysis of variance and determining the least significant difference in each set of data. Treatment means were compared to the respective mean of the control (hoeing) treatment.

RESULTS AND DISCUSSION

Weed Interference and Lettuce Growth. During the lettuce growing season (January–April) weed development in untreated plots was initially very slow. Weeds reached an appreciable growth to be regarded as competitive as late as the beginning of March (1986) or even the middle of March (1987). However, weeds grew fast thereafter, forming complete ground cover by normal harvest time (middle of April). The typical weed flora of the field was *Urtica urens* (60–80%), annual grasses such as *Avena sterilis* and *Phalaris brachystachys* (10–20%), and various annual broadleaves (*Raphanus raphanistrum*, *Sonchus oleraceus*, *Capsella bursa-pastoris*). Hoeing or any of the herbicides used gave excellent weed control, allowing only very few scattered weeds to survive; they thus practically removed weed interference from the crop.

Weed interference during the last 30–40 days of the 3-month growing period significantly reduced lettuce growth and dry matter accumulation (Table II). Fresh weight reduction rounded to about 50%, and dry weight reduction was about 25%. Hoeing and herbicides were equally effective in preventing weed interference effects on growth. Alachlor and pendimethalin, which are not as selective in lettuce as propyzamide, caused a slight retardation in the initial growth. This effect was evident at early stages of lettuce development but disappeared later. Lettuce plants grown under weed interference did not show any sign of degeneration at harvest, besides their smaller size, and looked marketable.

Nitrate Accumulation. Results from nitrate content analyses are shown in Table III. Values generally indicate nitrate levels in lettuce similar to those previously reported by other researchers (Siciliano et al., 1975) and relatively high to have a significant contribution to daily dietary intake of nitrates by humans (White, 1975). Significant differences are, however, observed among both weed control treatments and years. These differences can be of importance with regard to methemoglobinemia, especially in ethnic groups with high incidence of glucose 6-phosphate dehydrogenase deficiency.

Lettuce accumulated more nitrate in 1986 than in 1987, and this must be connected with some marked differences in weather between the 2 years (Table I). Weather during the growing period in 1987 was characterized by much higher rainfall, which possibly increased nitrate leaching and reduced the nitrate supply in the upper layer of the soil. Lower temperatures in 1987, on the other hand, may have negatively affected nitrate absorption by lettuce as it has been the case with spinach (Cantliffe, 1972).

Regarding nitrate level in headed lettuce, two different effects are observed by combined examination of data in Tables II and III: (1) A decreased nitrate content associated with decreased growth and dry matter accumulation resulted from weed interference in both years. It is noticeable that weed interference caused a 21–33% reduction in nitrate content of headed lettuce parallel to a decrease in both fresh (45–47%) and dry (24%) weight. (2) A relatively smaller decrease in nitrate content associated with a small increase of fresh weight (and no change of dry weight) was caused by some herbicides (propyzamide in 1986 and alachlor in 1987). The first effect apparently demonstrates how effectively weeds competed with the crop for available soil nitrate. The second effect is rather due to a simple dilution of nitrates by the herbicide-stimulated better growth, but it may be of practical importance if further substantiated.

Considering nitrate level in lettuce at a time before maturation (31 March 1987), another effect of herbicides can be observed (Table III). Lettuce grown in herbicide-treated soil had at this time much higher content of nitrates than same age lettuce grown in untreated soil. Herbicides thus acted by accelerating accumulation of nitrates to the level found in headed lettuce, as if inducing early maturation. Modification of root development by herbicides and nitrate acquisition [as discussed by Clarkson (1985)] from greater depth of soil, particularly in the rainy year of 1987, may be involved. This effect is undoubtedly of great interest and needs further elucidation.

Carotenoid Content. Lettuce carotene is known to be a mixture of three stereoisomers of β-carotene, namely neo-β-carotene B, *all-trans-β*-carotene, and neo-β-carotene U occurring at percentages of 5, 77, and 18%, respectively (Sweeney and Marsh, 1970; Rouchaud et al., 1984). Separation of the isomers in this study by TLC revealed that there were no qualitative differences among lettuce from the various weed control treatments, all containing the three isomers at about the above percentages. Total carotene content of lettuce was thus measured and is expressed as β-carotene in Table IV.

Lettuce xanthophylls include lutein, violaxanthin, neoxanthin, and β-cryptoxanthin at percentages of about 56, 26, 14, and 4%, respectively (Rouchaud et al., 1984). When xanthophylls were separated in this study by TLC on aluminum oxide, four components were detected in agreement with the above and no qualitative differences among treatments were observed. Relative abundance of total xanthophylls in lettuce from the various weed control treatments is shown in Table IV by the absorbance values at 445 nm of equivalent solutions.

All three carotene isomers of lettuce have provitamin A activity, whereas of the xanthophylls only β-cryptoxanthin possesses such activity [Zechmeister (1962) in Rouchaud et al. (1984)]. Since β-cryptoxanthin occurs at very low concentrations, changes in β-carotene concentration with the treatments directly reflect the changes of provitamin A value of lettuces.

Values for β-carotene content of lettuce obtained in this study varied considerably with year and treatment. Variation in xanthophyll content with treatment paralleled that of carotene.

Weed interference had the greatest effect on carotene content of lettuce, causing a reduction of 51–83% depending on the year. Reduction in carotene was 2–4 times higher than respective dry matter reduction, suggesting that carotene synthesis in lettuce is very sensitive to weed interference. These results point out the potential of weeds to diminish provitamin A value of lettuce and probably of other green vegetables and emphasize the importance of weed control in such crops.

Adverse weather conditions that prevailed in 1987 reduced both β-carotene content and dry matter accumulation to about the same percentage. Herbicides alachlor and pendimethalin, which did not affect carotene content of lettuce in 1986, caused 32 and 21% reductions, respectively, in 1987. These two herbicides are not as selective in lettuce as propyzamide. Rainy weather in 1987 may have facilitated root absorption of small quantities of the herbicides by the lettuce plants.

Propyzamide has been reported to slightly increase carotene content of lettuce (Rouchaud et al., 1984). No clear evidence was obtained in this study, but lettuce grown in propyzamide-treated plots was usually greener, contained more xanthophylls, and in 1986 had a significantly higher fresh weight than lettuce from other treatments (Table II).

Weed interference is shown by this study to have a great influence on nitrate and particularly on carotene accumulation in lettuce. With the exception of accelerated nitrate accumulation in premature lettuce, direct effects of herbicides were inconsistent and by far less important than their indirect effects through removing weed interference. The results prompt that weed interference should be seriously considered in future studies regarding quality and nutritional value of green vegetables.

Registry No. Nitrate, 14797-55-8; alachlor, 15972-60-8; pendimethalin, 40487-42-1; propyzamide, 23950-58-5.

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