Fertilization Effects on the Chlorophylls, Carotene, pH, Total Acidity, and Ascorbic Acid in Broccoli

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Waltham 29 broccoli was grown in soil 2 years with two sources of nitrogen (NO_3^- and NH_4^+), three levels of nitrogen (0, 50, and 100 pounds per acre), and two levels of iron chelate (0 and 10 or 15 pounds per acre). Nitrogen source had no effect on pH, total acids, chlorophylls, or carotene content of broccoli (1963). Application of nitrogen at the rate of 100 pounds per acre resulted in decreases in amounts of chlorophyll a, total chlorophyll, and carotene and in the percentage retention (pigment not converted to pheophytin) of chlorophyll a and total chlorophyll. Iron chelate applied at the rate of 15 pounds per acre reduced the ratio of chlorophylls a to b and decreased the percentage retention of chlorophyll b. Planting date and year effects resulted in more differences in the composition of broccoli than fertilization.

Plant pigments and their retention in processing are important factors in the quality of vegetables. Variation in the color of market fresh or frozen broccoli can be clearly observed. Degradation of chlorophylls during processing is affected by the presence of plant acids in the vegetable among other factors. This study was undertaken to determine the effects of nitrogen level, nitrogen source, and iron level in soil on chlorophylls a and b, carotene, pH, total acidity, and ascorbic acid in field-grown broccoli. The effects of planting date and year also were evaluated.

The importance of nitrogen level on the synthesis of chlorophylls in plants has been shown in sand culture (*6, 9*). Tam and Magistad (*12*) in a field study of pineapple plants found that, in general, $(NH_4)_2SO_4$ increased the chlorophyll in leaves, the increment being greatest when the original chlorophyll content of the leaf was lowest. In some cases, these authors noted decreases in chlorophyll with the application of excessive amounts of nitrogen.

Nitrogen source also has been found to affect chlorophyll as well as organic acids in plants. Sideris and Young (*11*) reported a higher chlorophyll content in pineapple plants grown in nutrient solution when NH_4^+ rather than NO_3^- ion was used. Vickery and coworkers (*13*) noted a marked decrease of organic acids in tobacco plants grown in culture solution when the proportion of NH_4^+ to NO_3^- ion was increased; however, a concomitant decrease in uptake of cations caused little change in free organic acids. Accumulation of plant acids is thought to be due to a block in one or more steps in the Krebs cycle (*2*) and the two predominant acids in broccoli have been found (*8*) to be citric and lactic in 3 to 2 ratio.

Chlorosis of leaves of plants grown in nutrient solution has been associated with iron deficiency (*14*). In sand cultures, the addition of iron has been found to increase chlorophylls and carotenoids in pineapple plants (*11*). Chlorosis also has been associated with high acid content of plants. Iljin (*5*) noted that large increases in citric acid occurred prior to visible chlorosis of the plant. Ascorbic acid has been reported to be decreased in the leaves of pineapple plants (*11*) when iron is added to the nutrient solution and the source of nitrogen is the NO_3^- ion.

Since most of the past studies on the effects of nitrogen and iron nutrition on chlorophyll and plant acids were carried out on nonedible plants grown in nutrient culture, the present experiment was undertaken to determine whether or not similar effects hold true for a field-grown edible plant.

Experimental

Waltham 29 variety of broccoli was grown in the falls of 1963 and 1964. In 1963, the treatments studied were two sources of nitrogen [$(NH_4)_2SO_4$ and $NaNO_3$], three levels of nitrogen (0, 50, and 100 pounds of nitrogen per acre), two levels of iron chelate (0 and 10 pounds per acre), two planting dates, approximately 2 weeks apart, and three replications ($2 \times 3 \times 2 \times 2 \times 3 = 72$). The 1964 treatments were the same as 1963 except that 15 pounds per acre of iron chelate and only one source (NH_4NO_3) of nitrogen were used ($3 \times 2 \times 2 \times 3 = 36$). In 1963, the whole plot was first fertilized with 500 pounds per acre of 8–16–16 fertilizer in order to provide sufficient nitrogen for growth on the check plots and adequate potassium and phosphorus for all plots. In 1964, potassium and phosphorus were provided by treatment of the whole plot with 400 pounds per acre of 0–20–20 fertilizer. The check plots thus received no nitrogen.

Samples of three to five heads of freshly harvested raw broccoli of about the same stage of maturity were analyzed for moisture, pH, total acidity, chlorophylls a and b, carotene, and, in addition, reduced ascorbic acid in 1964.

The methods used for moisture, pH, ascorbic acid, and chlorophylls have been reported (*3*). The AOAC (*1*) procedures were used for total acidity and carotene analyses. For carotene determination, maximum absorbance was found to be at 450 $m\mu$. At this wavelength the specific absorption coefficient of pure β-carotene (solvent 1 to 9 acetone-hexane) was 246.0.

Data were reduced by analysis of variance, correlation coefficients, the *t* test for unequal groups, and Duncan's multiple range (*7*).

Results and Discussion

Since the source of nitrogen, NH_4^+ or NO_3^-, was found to have no effect on any of the constituents under the conditions of the 1963 harvest, this treatment effect was eliminated in the 1964 study. Earlier work had reported an increase in chlorophyll (*11*) and a decrease in total acids (*13*) when the NO_3^- ion was replaced by the NH_4^+ ion as a source of nitrogen. However, these studies were carried out in nutrient solutions. Data from the present study do not confirm these effects in field-grown broccoli when plots are fertilized with 8–16–16 fertilizer prior to application of nitrogen.

Nitrogen level was found to have no effect on any of the constituents analyzed for broccoli grown in 1963 (Tables I, II, and III), but did show an effect in 1964 when there was no prior treatment of the plots with nitrogen. Added nitrogen at the 100 pounds per acre level was found (Tables II and III) to lower chlorophyll a, total chlorophyll, and carotene on the

Table I. Effect of Fertilization and Planting Date on Dry Matter, pH, Total Acidity, and Ascorbic Acid Content of Broccoli

(Moist basis)[a]

Factor[b]	Dry Matter, % 1963	Dry Matter, % 1964	pH 1963	pH 1964	Total Acidity, Meq./100 Grams 1963	Total Acidity, Meq./100 Grams 1964	Ascorbic Acid, Mg./100 Grams, 1964
Planting date							
Early	11.05[2]	11.09[2]	6.66[1]	6.63[2]	2.14[2]	1.69[2]	101.5
Late	12.65[1]	11.82[1]	6.56[2]	6.79[1]	2.60[1]	2.04[1]	104.8
Nitrogen level, lb./acre							
0	11.75	11.44	6.61	6.68	2.37	1.77	106.7
50	11.87	11.57	6.62	6.71	2.30	1.83	102.9
100	11.93	11.34	6.60	6.74	2.45	1.99	99.9
Iron chelate level, lb./acre							
0	11.65[2]	11.51	6.61	6.72	2.40	1.81	103.8
10–15[c]	12.05[1]	11.39	6.60	6.70	2.34	1.92	102.5

[a] 72 samples in analysis of variance in 1963, 36 in 1964.
[b] Means with different numerical superscripts are significantly different (7).
[c] Iron chelate added at rate of 10 lb./acre in 1963, 15 lb./acre in 1964.

Table II. Effect of Fertilization and Planting Date on Chlorophylls in Broccoli[a]

Factor[b]	Chlorophylls, Mg./100 Grams, Moist Basis: a 1963	a 1964	b 1963	b 1964	Total 1963	Total 1964	Chlorophylls a to b 1963	a to b 1964
Planting date								
Early	13.63[2]	11.00[2]	5.12	3.89	18.75[2]	14.86[2]	2.78	2.73
Late	14.82[1]	12.44[1]	5.29	4.11	20.11[1]	16.55[1]	2.66	3.06
Nitrogen level, lb./acre								
0	14.39	12.91[1]	5.24	4.30	19.63	17.20[1]	2.80	3.14
50	14.11	12.06[1]	5.20	3.95	19.31	16.01[1]	2.75	2.92
100	14.19	10.15[2]	5.18	3.75	19.36	13.90[2]	2.62	2.64
Iron chelate level, lb./acre								
0	13.95	11.61	5.11	3.98	19.06	15.59	2.82	3.15[1]
10–15[c]	14.50	11.80	5.30	4.02	19.81	15.82	2.63	2.65[2]

[a] 72 samples in analysis of variance in 1963, 36 in 1964.
[b] Means with different numerical superscripts are significantly different (7).
[c] Iron chelate added at rate of 10 lb./acre in 1963, 15 lb./acre in 1964.

Table III. Effect of Fertilization and Planting Date on Chlorophyll Retention (% Not Converted to Pheophytin) and Carotene Content of Broccoli[a]

Factor[b]	Chlorophyll Retention, %: a 1963	a 1964	b 1963	b 1964	Total 1963	Total 1964	Carotene, Mg./100 Grams Moist Basis 1963	Carotene 1964
Planting date								
Early	95.8	92.6	99.6	89.4	95.1	90.5	0.80[2]	0.62[2]
Late	96.5	94.0	91.2	95.2	94.2	93.5	0.96[1]	0.74[1]
Nitrogen level, lb./acre								
0	96.0	97.2[1]	97.3	100.2	94.9	95.8[1]	0.85	0.73[1]
50	96.3	94.9[1]	96.9	90.5	95.3	93.2[1]	0.90	0.71[1]
100	96.2	87.7[2]	91.9	86.3	93.9	87.1[2]	0.89	0.60[2]
Iron chelate level, lb./acre								
0	95.8	93.1	98.6	100.3[1]	95.1	93.7	0.85	0.68
10–15[c]	96.6	93.5	92.1	84.4[2]	94.3	90.4	0.90	0.68

[a] 72 samples in analysis of variance in 1963, 36 in 1964.
[b] Means with different numerical superscripts are significantly different (7).
[c] Iron chelate added at rate of 10 lb./acre in 1963, 15 lb./acre in 1964.

basis of milligrams per 100 grams fresh weight and also to lower the percentage retention of chlorophyll a and the total chlorophyll (that not converted to pheophytin). These effects on pigments were thought to be due to self-shading by the plants grown with high application of nitrogen. Tam and Magistad (*12*) had noted decreases in chlorophyll content of field-grown pineapple leaves when application of nitrogen was excessive. Previous work (*6*, *9*) had shown that a nitrogen deficiency in sand culture reduced the chlorophylls in plants. Results from the present study would indicate that, in addition to nitrogen deficiency, excessive application of nitrogen to soil also may reduce chlorophyll synthesis.

Application of iron chelate had an interesting effect on the synthesis of chlorophylls (Tables II and III). Although the amounts of the chlorophyll fractions (milligrams per 100 grams) were not affected by iron level, the ratio of chlorophylls a to b was decreased when 15 pounds per acre of iron chelate were applied in 1964. Ratios were calculated on the total amounts of the two fractions, a and b, present in the plant which included the chlorophyll plus any of the pigment which may have been converted to pheophytin. As can be seen in Table III, the percentage retention of chlorophyll b (amount not converted to pheophytin) was reduced from 100.3 to 84.4% by application of iron chelate (15 pounds per acre). The change in ratio was thus due to an increase in chlorophyll b by iron application but this increase did not show up in chlorophyll b (milligrams per 100 grams) because this calculation does not include pheophytin. A further calculation of chlorophyll b on the total basis (including the part converted to pheophytin) showed a highly significant increase of this fraction, from 4.17 to 5.02 mg. per 100 grams, by the application of iron chelate.

It is obvious from Tables I, II, and III that date of planting had a much more significant effect on constitutents analyzed than did either nitrogen or iron nutrition. Dry matter was higher in the late planting for both years. Total acids were higher in late than early planted broccoli in both 1963 and 1964, with a corresponding decrease in pH for 1963. The difference in total acids occurred when either the moist or dry basis was used for calculation. The pH of late broccoli was higher than early planted broccoli in 1964. The relationship between pH and total acids will be discussed later. Chlorophyll a, total chlorophyll, and carotene also were found to be higher in late planted broccoli both years when calculated on the moist basis. However, when these pigments were calculated on the dry basis differences were not significant. Thus the increase in these pigments with planting date was evidently due to the increase in solids.

Since planting date caused significant differences in certain constituents of broccoli, it seemed pertinent to test year effect. Total acids were higher and pH was lower in 1963 than in 1964 (Table IV). Broccoli grown in 1963 was higher in chlorophylls and carotene than that from the 1964 harvest. The ratio of the chlorophyll fractions did not differ significantly for the two years (2.72 in 1963 and 2.89 in 1964) and was not affected by planting date. Bonner (*2*) has stated that the ratio of chlorophylls a to b, although not constant, is thought to approach a value in plants of 2 to 3 to 1.

Planting date and year effects were thought possibly to be due to climatic factors—i.e., hours of sunshine and average temperature. However, correlation

Table IV. Year Effect on pH, Total Acidity, Chlorophyll, and Carotene Content of Broccoli

(Moist basis)

Component Analyzed	1963[a]	1964[b]	t^c
Dry matter, %	11.85	11.45	1.99 *
Total acidity, meq./100 grams	2.37	1.86	7.04 ***
pH	6.61	6.71	4.35 ***
Chlorophylls, mg./100 grams			
a	14.23	11.72	5.91 ***
b	5.21	4.00	8.27 ***
Total	19.43	15.70	6.84 ***
Chlorophylls, % retention			
a	96.2	93.3	2.35 *
b	95.4	92.3	0.78
Total	94.7	92.0	2.17 *
Chlorophylls a to b	2.72	2.89	1.46
Carotene, mg./100 grams	0.88	0.68	5.95 ***

[a] Means of 72 samples. [b] Means of 36 samples. [c] One and three asterisks indicate significance at 5 and 0.1% levels, respectively.

coefficients of pigments and plant acids with available weather data were inconclusive for this field study. Variable factors such as lag in effects of climatic conditions, plant age, and metabolism were probably involved in the failure to establish a relationship between climatic conditions and pigments or plant acids.

Total chlorophyll and carotene contents of broccoli had highly significant r values for both 1963 and 1964: 0.75 and 0.84, respectively. This relationship was reported earlier by Porter and coworkers (*10*), who found a ratio of chlorophyll to carotene in Swiss chard and beet greens of about 24 to 1. In the present study, the ratio for broccoli was 22 to 1 in 1963 and 23 to 1 in 1964.

Total chlorophyll and ascorbic acid contents of broccoli also were highly correlated with an r value of 0.51 for 1964. Therefore, the higher the chlorophyll content of the broccoli grown in this experiment, the higher were the ascorbic acid and carotene contents of the broccoli.

A highly significant r value (−0.74) for total acids and pH of broccoli was found in 1963. However, the 1964 value (0.15) was not significant. The relationship between total acids and pH of plants is not clear. Highly significant negative correlations between total acids and pH of lima beans have been found by Graham (*4*) in the author's laboratory. Sideris and Young (*11*) stated that although pH and acidity in pineapple leaves were in relative agreement, the presence of buffers made perfect agreement impossible. Bonner (*2*) has stated that a relationship between these two factors is not likely to occur, since pH is determined by pK_a plus amounts of free acids and their salts. Total acidity is a measure of only the free acids present.

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USE OF FORMAMIDE

Formamide as a Component of Liquid Fertilizers

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A MAJOR PROBLEM in production of liquid fertilizers is the limitation on nutrient content imposed by the water required to hold the salts in solution. A low nutrient content adversely affects costs of storing, handling, and shipping the product.

One major type of liquid fertilizer, nonpressure nitrogen solution, usually is prepared from aqueous solutions of urea and ammonium nitrate. The solubility of the urea and ammonium nitrate limits the nitrogen content of the solution to 32% at 32° F., 30.8% at 20° F., and 28.7% at 5° F. (*1*). Somewhat higher concentrations can be obtained by adding ammonia, but the resulting pressure introduces problems in handling, storage, and application to the soil.

A consideration of the properties of formamide led to a study of its suitability as an agent for raising the nutrient content of liquid fertilizers. Formamide, the acid amide of formic acid, contains 31% nitrogen. It compares favorably with urea in greenhouse tests (*2*). A liquid (f.p. 36° F.) with a low vapor pressure at ordinary temperatures, it is miscible with ammonia and water. It is a solvent for many fertilizer salts (*3*).

Salting-out temperatures of various fertilizer salts in formamide, or solutions containing formamide, were measured by cooling a solution of a salt to incipient crystallization, then warming it gently until the salt disappeared. The temperature of disappearance was taken as a conservative value for the salting-out temperature; this value is a little higher than the actual equilibrium temperature at crystallization point. Formamide was the practical-grade product, 30.6% N.