Nitrate Accumulation in Spinach Grown Under Different Light Intensities[1,2]

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Abstract. 'Winter Bloomsdale' spinach plants were grown in a growth chamber with a light intensity of 2400 ft-c until 2 weeks before harvest when light intensity treatments of 600, 1600, 2400, and 3500 ft-c were imposed. The total N and NO_3 concn of the leaves were increased by the addition of N to the soil and by reduction of light intensity. At 600 ft-c the plants accumulated NO_3-N and total N at all soil N levels, but response to soil increments of N was greater at higher light intensities. The concn of K in the tissue increased with a reduction in the light intensity from 2400 to 600 ft-c or application of N fertilizer to 200 mg/kg of soil. Less P was found in spinach leaves as N fertilizer was added to 100 mg/kg of soil. The P content was variable with light intensity.

Increased demands on our agricultural land to produce higher yields on less land has encouraged the use of large quantities of N fertilizer. This practice has its price: the possible accumulation of NO_3-N to high levels in plants used for consumption. The danger exists when NO_3 is reduced to NO_2. It is in this form that N enters the blood and forms lethal methemoglobin. Cases of NO_3 poisoning in animals (11, 24) and more recently in humans have been reported (8).

Fertilizer usage is not the only factor involved in this problem. Other factors, such as the environment (1, 2, 7, 10, 13, 15, 21, 23), certain herbicides (21, 24). insect damage (3), and general fertilizer practices (3, 4, 5, 10, 11) can all lead to the accumulation of NO_3 in the plant.

The accumulation of NO_3 in plants is generally dependent on the availability of soil NO_3 (2, 13, 23). Light has been shown to affect the NO_3 content of many plants (1, 4, 10) and has been correlated with nitrate reductase activity (2, 7, 13, 14, 15, 16, 17, 19, 23). In the North spinach is grown in the fall and spring months when the light intensity is relatively low. It is cropped at high densities, reducing light intensity by shading. The crop is often heavily fertilized with N to maintain green color, to improve quality and maintain yield. The combination of these factors can lead to high NO_3-N concn under field conditions. The concn of NO_3 in processed spinach is an important consideration especially with baby foods. It has been proposed that spinach for baby food should not contain more than 67 ppm NO_3-N on a fresh wt basis (20). The present study was initiated to determine the effect of light intensity and NO_3 supply on the accumulation of NO_3 in spinach.

Table 1. The effect of light intensity on the concn of various mineral nutrients in spinach leaves.[z]

Light intensity (ft-c)	Nutrient (% of dry wt)[y] NO_3-N	Total N	K	P	Dry wt g
		%			
3500	0.34a	3.53a	6.66a	0.90b	0.944a
2400	0.34a	3.35a	6.34a	0.79a	0.965a
1600	0.49b	4.24b	7.66b	0.76a	0.924a
600	1.26c	4.95c	9.92c	0.93b	0.901a

[z]Data are averages of all 6 N levels used.
[y]Means not following by the same letter are significantly different at the 5% level of confidence.

Materials and Methods

Spinach (*Spinacia oleracea* L. cv. Winter Bloomsdale) plants were grown in 12.5 cm plastic pots in an Ontario fine sandy loam soil in a quartered walk-in growth chamber. A soil test revealed a low total N content (0.13%) and 2.5% organic matter. The light intensity of the growth chamber was maintained at 2400 ft-c for 35 days after which the light intensity of each quarter of the chamber was changed to 600, 1600, 2400, or 3500 ft-c. The plants were harvested 2 weeks later. The temp of the chamber was maintained at 18°C day and 13°C night for the duration of the experiment. The photoperiod was 12 hr. At each of the 4 light intensities there were 6 N treatments equivalent to 0, 25, 50, 100, 200 and 300 mg N (as NH_4NO_3) kg of soil. The equivalent to 50 mg P/kg soil as Ca $(H_2PO_4)_2$ and 100 mg K/kg soil as K_2SO_4 were added to each of the pots. Each N treatment at each light intensity was replicated 4 times.

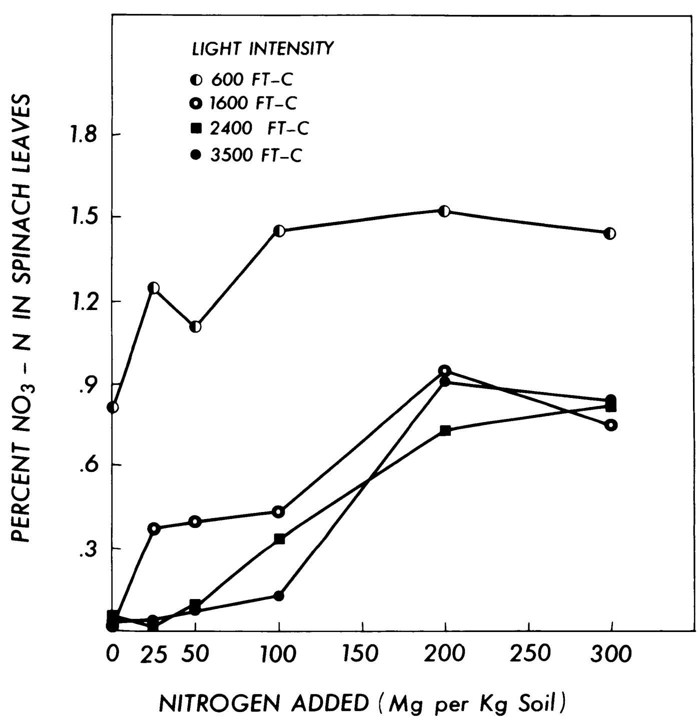

Fig. 1. Effect of light intensity and N supply on the NO_3 concn (dry wt basis) of spinach leaves.

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[4]Taber, H. 1969. The Accumulation of Nitrate in Tomato Fruit. M.S. Thesis, Purdue University, Lafayette, Indiana.

The plants were harvested 1 inch above the soil level, washed in distilled water, dried at 70°C for 48 hr, and then ground in a Wiley mill to pass through a 20-mesh screen.

The tissue was assayed for NO_3-N using an Orion Model 92-07 nitrate ion activity electrode (6). A portion of the plant tissue was wet digested using the nitric-perchloric acid digestion (9). Total N was determined by nesslerizing an aliquot of the digested sample (9, 18). Phosphorus was determined using the molybdovanado phosphoric acid procedure of Greweling (12) and K was determined by a Jarrel Ash 82-270 Atomsorb Atomic Adsorption Spectrophotometer using the flame emission mode. Statistical analyses of variance were performed and means were compared using Duncan's New Multiple Range Test (22).

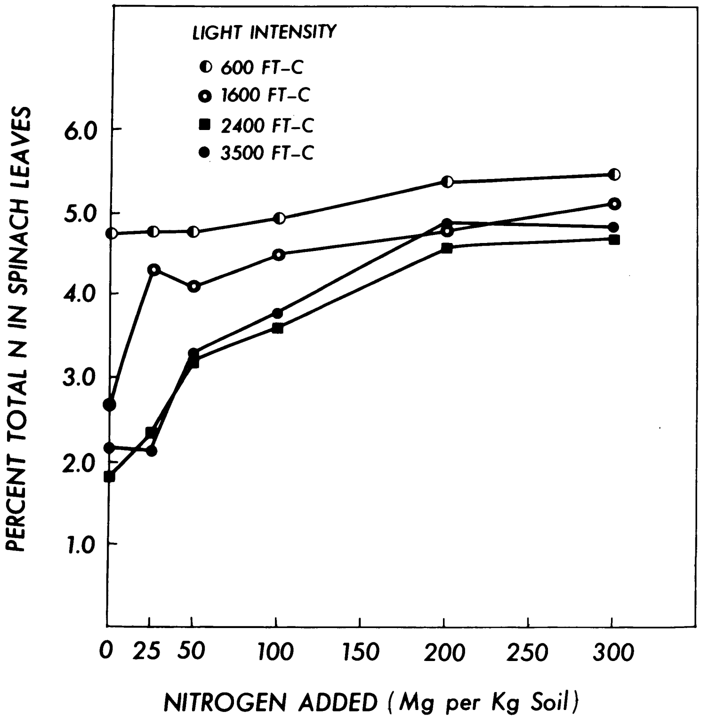

Fig. 2. Effect of light intensity and N supply on the total N concn (dry wt basis) of spinach leaves.

Results and Discussion

At light intensities of 2400 and 3500 ft-c there were no differences in the concn of NO_3-N, total N, and K in the tissue of spinach leaves (Table 1). However, these 3 consitituents appeared in higher concn at 1600 ft-c and still higher concn at 600 ft-c. Plants grown at 1600 ft-c had significantly more NO_3 and total N than plants grown at higher light intensities when fertilizer N was increased from 0 to 100 mg/kg soil (Figs. 1 and 2). The concn of NO_3-N and total N in plants grown at 600 ft-c were significantly higher at every fertilizer N rate than plants grown at any other light intensity. This accumulation was not due to differences in dry wt at the lower light intensities, since the plants were grown under a constant light intensity of 2500 ft-c until 2 weeks prior to harvest. Growth appeared normal and uniform at all intensities (Table 1). The concn of P in the tissue was greatest at 600 and 3500 ft-c. No satisfactory explanation for this variation can be offered. Reduction of light intensity has been shown to increase the P content of cacao (4) and tomato[4].

At light intensities of 3500, 2400, or 1600 ft-c 10 to 12% of the total N was NO_3-N (Table 1). But at 600 ft-c NO_3-N constituted 25% of the total N. An increase in the concn of total N in the tissue did not fully account for the increase in the concn of NO_3 in the tissue. Apparently NO_3 was not efficiently assimilated at the 600 ft-c light intensity.

Table 2. The effect of N on the mineral concn of spinach leaves.[z]

(Mg N/kg soil)	Nutrient (% of dry wt)[y] NO_3-N	Total N	K	P	Dry wt g
		%			
0	0.23a	2.84a	6.90a	1.08d	0.442a
25	0.42b	3.38b	7.44b	0.94c	0.795b
50	0.42b	3.83c	7.56b	0.86b	1.171de
100	0.59c	4.20d	7.78bc	0.71a	1.243e
200	0.97d	4.91e	8.22c	0.72a	1.107d
300	1.02e	4.93e	7.98bc	0.76a	0.857b

[z]Data are avg of all 4 light intensities at any 1 N treatment.

[y]Means not followed by the same letter are significantly different at the 5% level of confidence.

The accumulation of NO_3-N at low light intensity could result from decreased nitrate reductase activity (2, 7, 13, 14, 16, 23). Travis et al. (23) stated that when sufficient NO_3 was available, the extent of nitrate reductase activity was regulated by light. They found that NO_3 accumulated under conditions of low light and high NO_3 rates and attributed the NO_3 build up to light impaired nitrate reductase activity. Beevers et al. (2) described the role of light on NO_3 accumulation to be indirect in that light enhances NO_3 uptake as a result of increased permeability of the tissue. This in turn stimulates enzymatic activity and the assimilation of NO_3.

The NO_3-N and total N contents of the tissue increased as the amount of N added to the soil was increased to 300 mg/kg (Table 2). This response was greater at high light intensities than at 600 ft-c (Figs. 1 and 2). The K concn of the leaves generally increased with increasing applications of fertilizer N up to 200 mg/kg soil. The P content decreased with increasing additions of N fertilizer from 0 to 100 mg.

Spinach used for baby food should be grown with high light intensity to minimize NO_3 accumulation. Applying minimal rates of N fertilizer to spinach grown as a fall crop under continually decreasing light intensities may help to limit the build up of NO_3 without resulting in N deficiencies.

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Postharvest Benomyl and Thiabendazole Treatments, Alone and With Scald Inhibitors, to Control Blue and Gray Mold in Wounded Apples[1]

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Abstract. Benomyl and thiabendazole (TBZ), at concn of 1,000 ppm, were compatible with commercial scald inhibitors (2,700 ppm ethoxyquin or 2,000 ppm diphenylamine). No injury was observed on 'Delicious' or 'Stayman' apples given combined treatments and stored 5 months at 0°C plus 6 or 7 days at 21°C. Effectiveness of fungicide and scald inhibitor was not altered when combined.

Both benomyl and TBZ used as 10-15 sec dip treatments at 500 ppm controlled decay due to blue mold (*Penicillium expansum*) and gray mold (*Botrytis cinerea*) at puncture wounds in inoculated apples. They were less effective in controlling decay at bruises unless suspensions were heated in a range of 29°-45°C (84°-113°F) and used as a 2-min dip. Unheated benomyl was more effective than unheated TBZ in reducing blue mold at bruises. TBZ was less effective in controlling decay at punctures when treatment was delayed 24 hr after inoculation. TBZ added to water contaminated with blue mold spores, as in a dump tank, controlled decay at skin punctures but not at bruises during subsequent storage. Neither benomyl nor TBZ controlled Alternaria rot, which often developed at punctures when blue and gray mold rot were controlled.

Postharvest use of new systemic fungicides on apples offers excellent promise for reducing losses from blue mold (*Penicillium expansum* Lk. ex Thom) and gray mold (*Botrytis cinerea* Pers. ex Fr.), major causes of spoilage during storage and marketing in the USA. Now that apples are commonly dumped and handled in water and dunked or drenched with scald inhibitors, the chance of washing blue and gray mold inoculum into wounds is high. Blanpied and Purnasiri (1, 3) recently showed that the spore load in packing house water tanks is high and builds up as more and more apples are submerged. Recent examples of increased blue mold rot of apples following treatment in scald-inhibitor tanks have been published (6, 7).

Suspensions of 2-(4'-thiazolyl)benzimidazole (thiabendazole or TBZ) and methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate (benomyl) are being widely tested on horticultural crops. They appear to be more effective against blue mold than other commercial fungicides. Now that an experimental registration for use of benomyl on pome fruits has been issued, with a temporary residue tolerance of 7 ppm, commercial trial and usage may be rapid. TBZ, cleared for use on citrus and bananas, still awaits approval by the Environmental Protection Agency for use on apples. Unheated TBZ and benomyl apparently are harmless to apple skin and leave no visible residue. Neither material affects respiratory activity of apples (6).

Benomyl and TBZ control blue mold rot and some other rots in pears (9, 13) and apples (2, 4-7, 10, 11, 14, 15). For most effective decay control, fungicides should supplement good refrigerated storage. Both fungicides controlled blue mold on punctured and inoculated apples, but were less effective on bruised apples, unless suspensions were heated (14, 15). Bruising, which damages lenticels and leaves them open for infection, is an important factor in the development of blue mold rot (16). Heated TBZ or benomyl (3 min 45°C) were effective at concn of 100, 250, or 500 ppm in controlling blue mold of inoculated bruised apples without apparent injury (14). Dipping fruit for 45 sec in TBZ or benomyl heated to 54.5°C has sometimes caused a scald-like injury (15) similar to that found on apples treated with warm water for scald control (8). Treatment in 45°C water alone will control Gloeosporium rot of apples but increases physiological disorders (12).

Some data are available on effectiveness of TBZ and benomyl when applied with a scald inhibitor. Good decay control with combined treatments was obtained on 'McIntosh' (6) and 'Stayman' (7) apples, but the latter cv. was injured by fungicide-diphenylamine combinations.

Objectives of the current studies during 2 seasons were to determine effectiveness of benomyl and TBZ in controlling

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