Factors Affecting the Oxalate Content of Spinach

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The characteristic high oxalate content of spinach poses a serious nutritional problem because the calcium in the diet is made partially, and in some cases almost entirely, unavailable by the presence of oxalates. Statistically planned studies were made to determine the effect of such environmental factors as variety, soil moisture, light intensity, rate of growth, and time of harvest on the oxalate content of spinach. Since these environmental factors had very little effect on the oxalate content, it was concluded that the high oxalate content of spinach is an inherent physiological characteristic of this particular plant. Dry matter content and yield were affected by the aforementioned factors.

Nutritionally, spinach is a good source of minerals, vitamin B complex, ascorbic acid, and carotene (*20*); however, its high oxalate content often makes the calcium in the diet partially, and in some cases almost entirely, unavailable. This poses a nutritional problem of low calcium balances which very often results in toxic conditions.

When spinach was included in the diets of rats (*5, 7, 10, 16–18*), humans (*6, 17*), and ruminants (*17*), the calcium in the diet was partially or entirely immobilized. Only one case of a positive calcium balance for spinach-fed adults (seven healthy women) was found in this literature (*12*).

Soluble oxalates added to the diet in stoichiometric amounts for the calcium content of the diet were 100% effective in immobilizing the calcium when fed to 50-day old rats (*11*). When Ca^{45} oxalate was administered to mature and young rats, 20 and 44% of the calcium was fixed after 6 days (*9*). Oxalate toxicity was reported for sorrel-fed ewes (*2*). Human consumption of rhubarb leaves resulted in one death (*19*).

In a study (*1*) of the effect of light on acid plants, illuminated leaves produced oxalic acid only in the presence of carbon dioxide. Oxalic acid increases of 94, 64, and 57% were obtained when plants were exposed to daylight, red light, and blue light, respectively. Insoluble oxalate and insoluble calcium content of vegetables are highly correlated (*14*).

Studies of the chemical composition of plants indicate that such factors as variety, light intensity, soil moisture, time of harvest, and growth rate, result in differences in the chemical composition of plants. Very little work on the effect of these factors on the oxalate content of spinach is in the literature. Most of the reports on the oxalate content of plants do not indicate that statistically planned experiments were used to test the significance of the results. This paper reports the results obtained from statistically planned greenhouse experiments which were set up to study the effect of the aforementioned factors on the oxalate content of spinach.

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Experimental Procedure

For comparison, this study was carried out in two parts: Experiment I, a study of the effect of variety, soil moisture, and light intensity; and Experiment II, a study of the effect of variety, growth rate, and time of harvest. The effect of these factors on the oxalate and dry matter content and on yield was studied. A split-split plot design, with all the factors randomized, was used. The analysis of variance setup for both experiments is shown in Table I. Duncan's multiple range method (*3*) was used to show significant factor effect differences, Table III.

A sassafras sandy loam-type soil was used and was transported to Blacksburg from the Norfolk, Va., area. The greenhouse temperature for the growth period was controlled to correspond to daily 5-year mean temperatures at the Norfolk weather station. The soil type and the mean temperatures of the Norfolk area were used in an attempt to simulate optimum environmental conditions of a typical spinach-producing area.

The spinach was grown in greenhouse benches in rows 12 inches apart; the plants were planted 6 inches apart. An irrigation system, which watered the plants from underneath a black plastic mulch cover, rather than sprinkling them from above, was used. This prevented spattering the plants with soil. A 10–10–10 fertilizer at the rate of 800 pounds per acre was applied to all plants.

A satisfactory assay method was developed for oxalates and consisted of essentially the following three analytical steps: hydrochloric acid (3.5*N*) Soxhlet extraction of the dried, ground plant material; liquid-liquid ether extraction of a HCl-extract aliquot with subsequent precipitation of the oxalates as calcium

Table I. Analysis of Variance Setup for Oxalate-Spinach Study

Experiment I[a] Source	Degrees of freedom	Experiment II[b] Source	Degrees of freedom
Replicates (R)	3	Replicates (R)	3
Light intensity-50 and 100% (L)	1	Growth rate-rapid and slow (G)	1
Error A	3	Error A	3
Soil moisture-low and high (M)	1	Varieties (V)	7
L × M	1	V × G	7
Error B	6	Error B	42
Variety (V)	11	Harvest-early and late (H)	1
V × L	11	H × G	1
V × M	11	H × V	7
V × L × M	11	H × V × G	7
Error C	132	Error C	48
Total	191	Total	127

[a] Twelve varieties × 2 soil moistures × 2 light intensities × 4 replicates = 192. Split-split plot design. All factors randomized.
[b] Eight varieties × 2 growth rates × 2 times of harvest × 4 replicates = 128. Split-split plot design. All factors randomized.

oxalate; and $KMnO_4$ titration of the calcium oxalate. A detailed description of this method, with statistical tests for its precision, is given in another publication (*4*).

Experiment I. Twelve standard varieties were used and the effects of 50 and 100% light intensities were studied. The intensity on the 50% plots was controlled with cheese cloth covers placed approximately 1 foot above the plants, and extending down to the bench on all sides. Light intensity was measured with a Weston light meter. The 100% plots were left uncovered and exposed to full sunlight.

High and low soil moistures consisted of 1 and 4 atmospheric tensions of pressure, respectively. Two tensiometers were used in each individual plot (3 × 6 foot) to determine when moisture was needed in the high-moisture plots. Five gypsum blocks were placed in each of the low-moisture plots (3 × 6 foot), and a Bouyoucos moisture meter was used to determine when moisture was needed on these plots. The gypsum blocks gave more precise measurements on the low-moisture plots, and the irrometers were more precise for the high-moisture measurements.

The plants were harvested when they attained commercial maturity. Three-plant composites from each variety for each plot were analyzed for oxalic acid and dry matter. Individual plants were weighed for yield.

Experiment II. The procedure for this study was similar to that of Experiment I. The same soil, fertilization, irrigation method, mulching, harvesting, and sampling techniques were used. Three standard varieties, two seedlings, and three plant introductions (Table III), were used to study the generic effect on the oxalate and dry matter content and on the yield. Two new factors were introduced: rapid and slow growth, and early and late harvests (Table I).

Rapid growth was attained by regulating one greenhouse unit at 65° to 75° F.; slow growth was attained by regulating another unit at 45° to 55° F.

Previous research (*8*, *13*) indicated that the level of maturity influenced the oxalate content of plants. The effect of this factor was studied by making an early and late harvest on both the rapid and slow growing plants. The early harvest was made when the plants attained proper size for commercial use; the late harvest was made 2 weeks later. The introduction of this factor into the study should give some indication of maturity effects.

Table II. Significant Factor Effects for Oxalate-Spinach Study

	Oxalic Acid		Dry Matter,	Fresh Weight Yield,
	W. B., %	D. B., %	%	Lb./Acre
Experiment I				
Varieties	[a]	[a]	[a]	[a]
Experiment II				
Growth (G)			[a]	[a]
Varieties (V)		[b]	[a]	[a]
V × G			[a]	[a]
Harvest (H)	[b]		[a]	[a]
H × G		[a]	[a]	[a]

[a] Significant at the 1% level.
[b] Significant at the 5% level.

Table III. Significant Variety Effects for Oxalate-Spinach Experiments

Varieties	Oxalic Acid, Wet basis, %	Oxalic Acid, Dry basis, %	Dry Matter, %	Fresh Weight Yields, Lb./Acre
EXPERIMENT I				
Early hybrid No. 7	1.15[bcd]	10.92[abc]	10.51[bcd]	18,784[bc]
Virginia Savoy	1.10[def]	10.46[cd]	10.40[bcd]	8,069[fg]
Old Dominion	1.09[def]	10.65[cd]	10.18[cd]	9,208[fg]
Bloomsdale Long Standing	1.10[def]	10.93[abc]	10.00[de]	7,360[gh]
Hi Curl Savoy	1.05[f]	10.46[cd]	9.98[de]	9,902[efg]
America	1.08[ef]	10.30[d]	10.54[bc]	5,316[h]
Dixie Market	1.03[f]	10.59[cd]	9.66[e]	12,304[de]
Viroflay	1.23[a]	11.06[abc]	11.17[a]	20,462[b]
Viking	1.15[bcd]	10.76[bcd]	10.72[ab]	10,333[ef]
Giant Nobel	1.18[ab]	11.30[ab]	10.36[bcd]	23,551[a]
King of Denmark	1.16[bc]	11.07[abc]	10.47[bcd]	17,291[c]
Troubador	1.24[a]	11.38[a]	10.76[ab]	14,082[d]
Mean	1.13	10.82	10.40	13,055
Range	1.03–1.24	10.30–11.38	9.66–11.17	5,316–23,551
EXPERIMENT II				
Early hybrid No. 7		10.43[a]	9.75[cd]	15,589[ab]
Old Dominion		10.04[a]	9.27[d]	8,636[d]
Giant Nobel		10.09[a]	9.62[cd]	10,141[cd]
Webb-214		10.52[a]	9.63[cd]	10,775[cd]
Webb-384		10.34[a]	8.73[d]	11,351[c]
P. I. 175–385		9.20[ab]	10.47[a]	16,562[a]
P. I. 176–371		8.66[b]	9.94[bc]	14,379[ab]
P. I. 176–772		9.63[ab]	10.40[ab]	13,854[b]
Mean		9.87	9.73	12,660
Range		8.66–10.52	8.73–10.47	8,636–16,562

[a–g] "a" data are significantly greater than those of "b," "b" data are significantly greater than those of "c," etc. (*3*).

Experimental Results

Experiment I. Variety was the only factor studied which had any statistically significant effect (Table II). Light intensity, soil moisture, and all interactions were not statistically significant. The lack of a light intensity effect was not in agreement with Bassalik (*1*), who found that when leaves of acid plants were exposed to daylight, an increase of 94% in the oxalic acid content occurred; but this was in agreement with Pucher, Wakeman, and Vickery (*15*), who found that the oxalic acid content of tobacco leaves changed very little when exposed to light.

Table III gives the oxalic acid content on the wet and dry basis, the dry matter content, and the yield in pounds per acre for the 12 standard varieties studied. The means, ranges, and significance are also given. The greatest range was obtained with yield. America was the lowest yielding variety with 5316 pounds per acre, while Giant Nobel was the highest with 23,551 pounds. The differences in dry matter and oxalic acid content, although statistically significant, were not of sufficient magnitude to be of any practical importance.

Experiment II. Reference to Table II shows that statistically significant factor effects were obtained with growth rate, variety, and harvest date. Variety × growth rate and harvest × growth rate interactions were also significant.

Table III gives significant data for the different varieties studied. Plant Introduction (P. I.) No. 176-371 was significantly lower in oxalic acid content, 8.66%, than the three standard varieties and the two seedlings, but was not lower than the other two plant introductions. The difference in dry matter content of the varieties, although significant, was not large enough to be of importance. Old Dominion was the lowest yielding variety, 8636 pounds per acre, while P. I. 175-385 was the highest, 16,562 pounds. This constituted an important difference of 92%.

The early and late harvest effects on oxalic acid and dry matter content, although significantly different, were not of sufficient magnitude to be important. The yield of the late harvest, 16,581 pounds per acre, was 90% higher than that of the early harvest plots, 8739 pounds, and this was of practical importance.

The dry matter content of the rapid growth plants was 8.81%, while that for the slow growth ones was 10.64%. The slow growth yield was 5442 pounds per acre, and the rapid growth yield was

19,789—a large difference of 364%.

Significant interaction data for variety × growth rate for dry matter and yield, and harvest × growth rate for oxalic acid and dry matter content and yield are not given because their inclusion would not have added any pertinent information.

Assuming the possibility of lowering the oxalate content by crossbreeding, a study of this type was initiated. When a number of foreign introductions were screened for oxalate content, several proved to be low in oxalates. These low-oxalate introductions were crossed with standard varieties in an attempt to lower the oxalate content of the latter. This work is just getting under way, and no conclusive results have been obtained at this time.

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NUTRIENTS IN MARKETED FERTILIZERS

Calcium, Magnesium, and Sulfur Contents of Mixed Fertilizers Marketed in 1949–50 and in 1955–56

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The trend to higher analysis fertilizers often is regarded as signifying an accompanying decrease in the calcium, magnesium, and sulfur contents of the nation's fertilizers. These secondary elements were determined on 425 and 491 samples, of mixed fertilizers marketed during the 1949–50 and 1955–56 fertilizer seasons. Although the average calcium, magnesium, and sulfur contents decreased 5.3, 12.8, and 4.8%, respectively, in this 6-year interval, they remained at substantially the same levels as in earlier years. The ratios of the secondary nutrients to nitrogen, phosphorus, and potassium decreased appreciably, however, because of the trend to high-analysis mixtures. The tonnage of secondary elements applied in mixtures during the 1955–56 season exceeded that applied in the 1949–50 season, since the increase in tonnage of marketed fertilizers more than offset the decrease in the average contents of the secondary elements.

The trend to higher analysis fertilizers often is regarded as signifying an accompanying decrease in the calcium, magnesium, and sulfur contents of the nation's fertilizers. Since soil additions of these elements are needed in many areas, decreases in the quantities present in fertilizers may result in their inferior performance under crops. Reliable information, therefore, is needed on this aspect of fertilizer composition.

Surveys of solid mixed fertilizers marketed in the United States during the 1949–50 and the 1955–56 fertilizer seasons were conducted to obtain information on their physical and chemical characteristics (*1–4*). Thus, 916 mixed fertilizers, representative of technological conditions at the time of their production, were available for determining the trend in secondary nutrient content over the 1949–1955 period. The 425 samples collected in 25 states for the 1949–50 survey represented the products of 157 manufacturers and were marketed in 91 grades and 58 plant-nutrient ratios. Of these samples 23 were N-P, five N-K, 24 P-K, and 373 N-P-K mixtures. The 491 samples collected in 35 states for the 1955–56 survey represented the products of 160 manufacturers and were marketed in 90 grades and 60 plant-nutrient ratios. Of these samples, 17 were N-P, five N-K, 26 P-K, and 443 N-P-K mixtures.

Analytical Methods

Solutions for the determination of calcium, magnesium, and sulfur were prepared by digesting a portion of the ground fertilizer with a 3 to 1 concentrated nitric-hydrochloric acid mixture.