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Nitrate Accumulation in Vegetable Crops as Influenced by Soil Fertility Practices

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J. R. Brown and G. E. Smith

INTRODUCTION

Nitrates have been a newsworthy topic for over a decade as a result of the association between nitrate intake and animal performance. The accumulation of nitrate in plants has been blamed, in part, upon fertilizer.

Nitrate accumulation in plants, to a certain extent, is a natural and necessary process. Nitrate is a form of nitrogen which is readily available in the soil for use by plants. Most plants take up nitrate preferentially over other forms of nitrogen such as ammonium. Once nitrate enters a normally functioning plant, incorporation of the nitrate ion into amino acids and proteins by reduction and combination with products of photosynthesis takes place. Any interference with this process of synthesis will cause nitrate accumulation. For example, dry weather or shading slows photosynthesis, which lowers the quantity of available photosynthate, causing accumulation of nitrate.

Unfortunately, nitrate accumulation in plants may be caused by too much fertilizer nitrogen being applied to the soil. That is, the nitrate supply in the plant may exceed the capacity of that plant to synthesize useful nitrogen products. The data reported in this bulletin resulted from investigations on the relationship between fertilizer practices for vegetables and nitrate accumulation.

REVIEW OF LITERATURE

Most of the work on nitrate accumulation in plants has been done with field crops and forages. An excellent review of the nitrate problem was published in 1964 by Wright and Davison (20). No attempt will be made to duplicate their review; instead, a brief summary of the work reviewed by these workers follows:

1. Small but consistent differences have been observed in nitrate accumulation among species of the same variety.
2. In general, stems are higher in nitrate content than leaves or reproductive organs; the lower stem is generally highest in nitrate content.

3. The nitrate content of plants generally reaches a maximum in the prebloom stage of growth and then declines with increasing maturity. This phenomina has been attributed to:
   a. The changing proportions of stem, leaf and fruit with increasing maturity which may cause dilution of nitrate as fruits are always low in nitrate compared to the remainder of the plant.
   b. Fruit or seed formation which creates a heavy demand for nitrogen.
   c. The nitrate supplying capacity of the soil which diminishes as the season progresses.
4. Shading and cloudiness may cause nitrate accumulation.

As pointed out earlier, the review by Wright and Davison concern mainly field and forage crops. Actually, little work has been reported on the accumulation of nitrate in vegetable crops. Wilson (17, 18) reported on some of his work with vegetables, where he found large accumulations of nitrate in certain vegetables, but he did not relate these nitrate contents directly to either fertility practices or to effects upon humans who might consume this material. Gilbert *et al.* (9) also have reported that various vegetables accumulate nitrate. In addition, Gilbert *et al.* (9) reported that a toxic dosage of nitrate for humans was 4 grams of KNO_3 daily (0.56 g NO_3-N).

Whitehead and Moxon (16) reported that the toxic level of nitrate for cattle was 1 g KNO_3 (0.13 g NO_3-N) per kilogram of body weight. At this rate of nitrate intake, 70 to 80 percent of the hemoglobin in the blood of the animal is changed to methemoglobin in a few hours and is fatal. Nitrate poisoning results in formation of abnormal quantities of methemoglobin in the blood which cannot release oxygen to the cells of the body. Lack of oxygen causes a form of asphyxiation which is manifested in cyanosis in human infants (blue babies).

While the quantitative values of toxic levels of nitrate given above have been reported, no good documentation of the values exists. Comly (5) documented the cases of several blue babies but gave no good guides on the relationship between nitrate intake and cyanosis. Sollman (14) reports that 5 grams of potassium nitrate at one dose may be dangerous and 8 grams may be fatal. If nitrate is ingested, Sollman (14) states that 2.6 grams of potassium nitrate can be fatal. Walton (15) reported that infant formula should not exceed 10 or possibly 20 ppm NO_3-N. Wright (19) gives the U.S. Public Health Service standard for drinking water as a maximum of 45 mg. of nitrate per liter.

Workers in the animal husbandry and agricultural chemistry departments at the University of Missouri have shown that nitrates tend to increase thyroid gland size and reduce Vitamin A storage in certain animal species such as rats and sheep (2, 8, 13). These results would indicate that nitrate may influence the physiology of the animal in several ways.

The literature is quite confusing in respect to the method of reporting nitrate content. Nitrate may be expressed as percent, parts per million, or grams per kilogram. Bloomfield *et al.* (1) give conversion tables for placing nitrate contents on a single basis of calculation for comparative purposes. When the above data from the literature is converted to the nitrate-nitrogen form the following summary may be made.

Conditions	NO_3-N	Source
Toxic daily dose-humans	0.56 g	Gilbert et al (9)
Toxic dose-cattle	0.13 g/kg body weight	Whitehead and Moxon (16)
Possible toxic single dose-humans	0.70 g	Sollman (14)
Infant formula concentration-Maximum	20 ppm	Walton (15)
U.P.H.S. water standard	10 ppm	Wright (19)

Unfortunately, no clearer definition of toxic levels of nitrate can be obtained readily from the literature. In addition, no really extensive study of the relationship between fertility practices and nitrate accumulation in foodstuffs has been made. This study was undertaken with the support of the U.S. Public Health Service to provide more information. A preliminary report has been published (4).

METHODS

The study was initiated in 1963 and was continued in the two succeeding years. The vegetables were grown on a site predominately of Mexico silt loam on the Clay Pan Experiment Farm at McCredie. The area selected was divided into three replicates, 70 x 117 feet. Soil samples were taken just after plowing under the bluegrass sod in March, 1963. The initial soil test results are given in Table 1.

TABLE 1--INITIAL SOILS TEST RESULTS; VEGETABLE PRODUCTION AREA, CLAYPAN EXPERIMENT FARM, McCREDIE, MO.*

Replicate	O.M.	P_2O_5	K	Mg	Ca	pH_W	pH_S	Exch H
	%	ppm of Soil						me/100g
1	3.0	16	65	600	3900	5.7	5.3	3.0
2	3.3	11	90	580	3350	5.6	5.2	4.0
3	2.8	10	85	690	2650	5.6	5.1	4.5

*Tests made as outlined in Missouri Bulletin 734 (10).

Each replicate was divided into nine 13 x 70 foot plots. Each plot within each replicate was randomly assigned one of the nine fertilizer treatments outlined in Table 2. The fertilizer was applied with a 10-foot fertilizer spreader which left 1½ feet of untreated border on each side of each plot.

TABLE 2--FERTILIZER TREATMENTS, VEGETABLE STUDY AREA
CLAYPAN EXPERIMENT FARM, McCREDIE, MO.*

Treatment	Nitrogen**	P_2O_5	K_2O	Ca***	Treatment Code
	lbs/A				
1	0	200	100	3200	0
2	50	200	100	3200	50
3	100	200	100	3200	100
4	200	200	100	3200	200
5	400	200	100	3200	400
6	200	0	100	3200	$200P_0$
7	200	200	0	3200	$200K_0$
8	200	200	100	0	$200L_0$
9+	200	200	100	3200	200T

*Nitrogen as NH_4NO_3, P_2O_5 as 45% superphosphate, K_2O as 60% KCl and Ca as $Ca(OH)_2$.

**Nitrogen was applied in 1964 and 1965 at one half the 1963 rate.

***Calcuim was applied only in 1963.

+Treatment 9 consisted of a micronutrient mixture in addition to the base treatment. In 1963 this mixture was 7.6 lbs. Cu, 2.3 lb. Zn, 1.1 lb. B, 1.2 oz. Mo. and 0.5 oz. Co. per acre. Only the Mo was applied in 1964. Micronutrients were not applied in 1965.

Several different species of vegetables were selected for study. The species selected were those that could be planted at the same time in the spring to facilitate field work. For most species, two or more varieties were selected to measure the varietal differences in nitrate accumulative ability.[1] Table 3 lists the species and varieties used in this study, as well as the years in which these varities were used. The changes in varieties used resulted from lack of seed, from poor growth the previous season, or from additions to the size of the study. A fall crop was grown in 1964 in an attempt to measure the seasonal influence on nitrate accumulation.

Vegetables were planted in 40-inch rows across the fertility treatments. Species were assigned to rows at random within each replicate and all varieties of each species were planted in adjacent rows. The plots were sprinkler-irrigated when moisture was needed, as determined by visual inspection.

Each vegetable treatment combination was sampled by obtaining a grab sample from the center of each treatment area when the respective vegetables making the best growth were near marketable size. The samples were placed in polyethylene bags for freezing and were stored in a local cold storage plant until they were analyzed for nitrate content. For vegetables such as lettuce, that might be harvested several times in a home garden, samples were taken two or, in some cases, three times to determine the trend in nitrate accumulation. The dates of

[1] Seed was donated by the Corneli Seed Co., St. Louis, Missouri.

TABLE 3--SPECIES AND VARIETIES USED IN THE VEGETABLE STUDY 1963 - 1965

Common Name	Botanical Name	Variety	Year Used
Radish, Red	Raphanus sativus	Champion	63, 64, 65
		Scarlet Globe	63, 64, 65
		Cherry Belle	63, 64 F*
		French Breakfast	64 F*
Radish, White	Raphanus sativus	Icicle	63, 64, 64F, 65
Turnip	Brassica rapa	Shogoin	63, 64, 65
		Purple top white globe	63, 64, 64F, 65
Beet	Beta vulgaris	Detroit dark red	63, 64, 64F, 65
		Early wonder	63, 64, 65
		Blood Turnip	64F
Carrot	Daucus carota	Nantes	63, 64, 65
	var. sativa	Chantenay Red Core	63, 64, 65
Mustard, leaf	Brassica junicea	Green wave	63, 64, 65
Mustard, spinach	Brassica perviridis	Tendergreen	64, 64F,65
Mustard, curled	Brassica junicea		
	var. crispifolia	Southern Giant curled	
Lettuce, leaf	Lactuca sativa	Blackseeded Simpson	63, 64, 64F, 65
	var. crispifolia	Salad bowl	63, 64, 64F, 65
Lettuce, head	Lactuca sativa	Bibb	63
Lettuce, head	var. capitata	Buttercrunch	63, 64, 65
Kale	Brassica oleracea	Dwarf Blue Scotch	63, 64, 65
	var. acephala	Siberian Improved	63, 64, 64F, 65
Snapbeans	Phaseolus vulgaris	Contender	64F,65
Swiss Chard	Beta vulgaris var cicla		
Spinach	Spinacia oleracea	Bloomingsdale	
		" Long standing	63, 64, 64F, 65
		" Dark Green	63, 64, 64F, 65

* F refers to the fall crop.

sampling are given in Table 4. The planting dates were: April 12, 1963; April 16 and 17, 1964; August 7, 1964, and April 23, 1965.

TABLE 4--DATES OF SAMPLING OF VEGETABLES IN THE VEGETABLE STUDY

1963-1965 Vegetable	Season 1963	1964 Spring	1964 Fall	1965
Radish, red	11 May 21 May	21 May	4 Sept	21 May
Radish, white	18 May	23 May	4 Sept	28 May
Turnip tops	29 May	16 June 14 July	24 Sept	29 June
Turnip roots	17 June 26 June	24 June 14 July	None	29 June
Beets	26 June	26 June 14 July	None	25 June
Carrot	16 July	17 July		29 July
Mustard	28 May	9 June 22 June	24 Sept	5 June
Lettuce, leaf	30 May 10 June	3 June 16 June 24 June	29 Sept	8 June
Lettuce, head	24 June	2 July	None	None
Kale	31 May 10 June	2 July	29 Sept	17 June 25 June
Snapbeans			29 Sept	25 June
Swiss Chard	None	4 June		15 June
Spinach	10 June	4 June	None	5 June

The frozen plant material was homogenized in a Waring blender and nitrate was extracted from the homogenate with hot KCl solution. The nitrate nitrogen concentration of the extract was determined by reduction with Devarda's alloy under steam by a modification of the procedure described by Bremmer (3) and Hanway *et al.* (12). All data, unless otherwise indicated, is presented as percentage nitrate nitrogen on the dry matter basis.

Satistical analyses of the data were performed according to procedures outlined by Federer (7). Tests were made on the means by the Duncan New Multiple Range procedures (6).

RESULTS AND DISCUSSION

General

The preceding section has indicated the complexity of this study. In an attempt to present the results in a simple manner, this section will be divided into subsections, each of which will be devoted to a specific vegetable species. Root crops will be discussed first, followed by the leafy vegetables.

As explained in Table 2, the nitrogen rate used in 1963 was twice the rate used in 1964, and 1965. The treatment code presented in Table 2 will be adhered to in the tables and discussion to follow. The reader should keep in mind the difference in rates of nitrogen used in the various years of the study.

Red Radish

A few varieties were used all three years of the study, enabling use of a statistical approach to give an indication of an effect of the different years if such an effect was present. Champion and Scarlet Globe were two varieties of red radishes which were used in all three years. The results of the statistical analysis of the nitrate nitrogen contents of these two varieties (Table 5) indicate an absence of significant interactions suggesting that both radish varieties responded similarly to the fertility treatments all three years.

TABLE 5--ANALYSIS OF VARIANCE OF THE NITRATE NITROGEN CONTENT OF TWO VARIETIES OF RED RADISH GROWN ON DIFFERENT FERTILITY TREATMENT IN 1963, 1964 and 1965

Source	df	m.s.	Level of significance
Year (y)	2	3.2094	5%
Replicate in year	6	0.5693	5%
Treatment (Tmt)	8	0.6021	5%
Error A	64	0.2283	-
Variety (V)	1	1.0161	5%
Variety x treatment	8	0.1627	NS
Variety x Year	2	0.4071	NS
V x Y x Tmt	16	0.1548	NS
Error B	54	0.1804	-
Total	161	-	-

The overall mean nitrate nitrogen contents of the Champion and Scarlet Globe varieties were 1.01 and 0.85 percent, respectively. The two means are significantly different at the 5 percent level, indicating that under the conditions of the experiment, varieties of radishes differ in their nitrate contents. Work with other crops by workers at other stations give indications that the ability of a plant to accumulae nitrate or to prevent accumulation may be inherited [eg. Hageman (11)].

Table 5 showed that the fertility treatments had a significant influence upon the accumulation of nitrate in the two varieties of red radish. Table 6 presents a list of means by treatment which provides a detailed view of which treatments were actually responsible for significantly different plant nitrate contents. The means show that the highest nitrogen rate resulted in the highest nitrate content in the radishes. Table 6 also indicates that the omission of phosphorus seemed to significantly reduce nitrate accumulation. This observation must be weighed in light of field observations.

TABLE 6--MEAN NITRATE NITROGEN CONTENTS OF RED RADISHES GROWN IN 1963, 1964, and 1965 UNDER DIFFERENT FERTILITY TREATMENTS

Treatment*	Mean** % NO_3-N
0	0.75^{bc}
50	0.87^{bc}
100	0.87^{bc}
200	1.04^{ab}
400	1.22^{a}
$200P_0$	0.59^{c}
$200K_0$	1.03^{ab}
$200L_0$	0.97^{ab}
200T	1.02^{ab}

*See Table 2 for the full explanation for the treatment code.

**Means carrying the same letter superscript are not significantly different at the 5% level according to the Duncan's Multiple Range Test (6).

The spring of 1964 was wet and cool. These weather conditions resulted in two phenomena which had profound influence upon the accumulation of nitrate in the radishes and were actually responsible for the significant year effect shown in Table 5. First, the weather was conducive to the infestation of the radishes, as well as other vegetables, by aphids. Control measures were not effective because of frequent rainfall which seemed to remove the insecticide almost as soon as it was applied. Second, the cool weather restricted mineralization of phosphorus from the soil organic matter and caused a severe phosphorus deficiency on the plots which received no phosphorus fertilizer. This phosphorus deficiency was so intense that the radishes planted on these plots did not grow. Consequently, no samples were obtained. To facilitate statistical analysis the nitrate contents of these plots were recorded as zero, thus negating any observations that might be drawn from the $200P_0$ mean in Table 6. However, the mean nitrate nitrogen content of radishes grown in 1963 and 1965 on the $200P_0$ plots was 0.79 percent, which is well below the 200 treatment mean of 1.04 percent.

The insect problem of 1964 seemed to be responsible for the significant difference in nitrate content between years. The mean nitrate contents were 0.78, 1.21, and 0.79 percent in 1963, 1964, and 1965, respectively. Wright and Davison (20) report that no evidence was found in the literature concerning the effect of parasites on nitrate accumulation in plants, but it is logical to assume that when the parasites (aphids) remove plant food from the plant nitrogen metabolic systems within the plant the metabolism of nitrate could be affected.

The higher nitrate content of the radishes in 1964 might have been influenced by the cloudy, cool weather which would be conducive to nitrate accumulation. In any event, the conclusion can be reached that any environmental condition that is not ideal for plant growth could contribute to nitrate accumulation.

In spite of the insect problem in 1964, the influence of increasing rates of nitrogen fertilizer upon nitrate accumulation in the two radish varieties is of interest (f. Figures 1 and 2). The insect problem of 1964, alone, might be sufficient to require consideration of the data from each year. However, another reason for individual year consideration resulted from attempts to maximize the yield of information from this study. These attempts included addition or deletion of varieties, a time of sampling study, and a part-of-plant study.

Figure 1. Percentages of nitrate nitrogen in Champion red radish fertilized with various rates of nitrogen fertilizer.

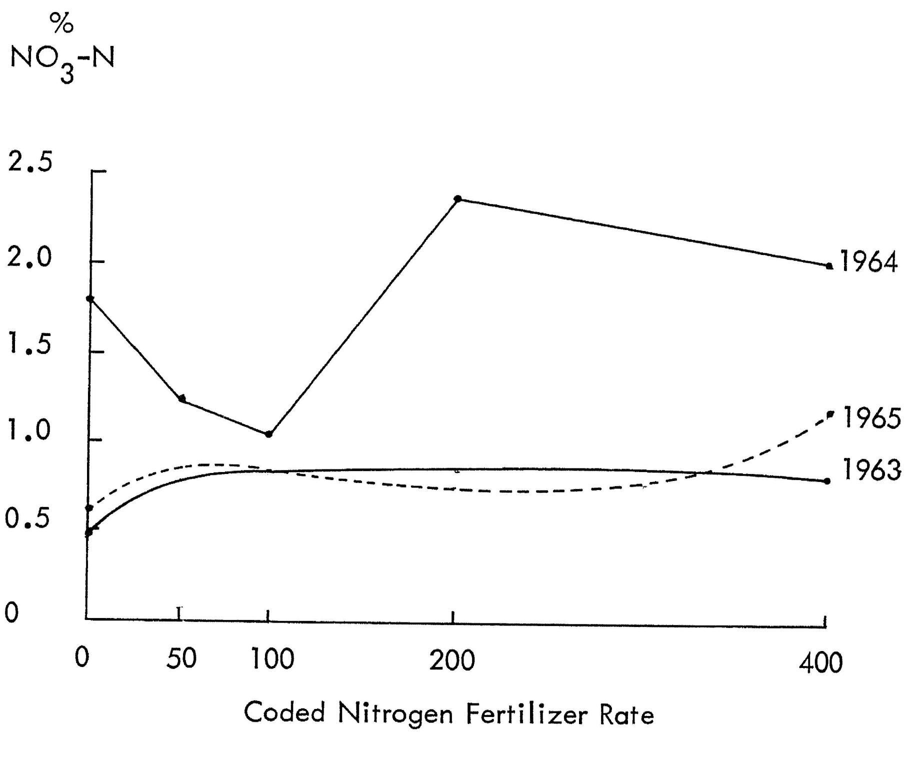
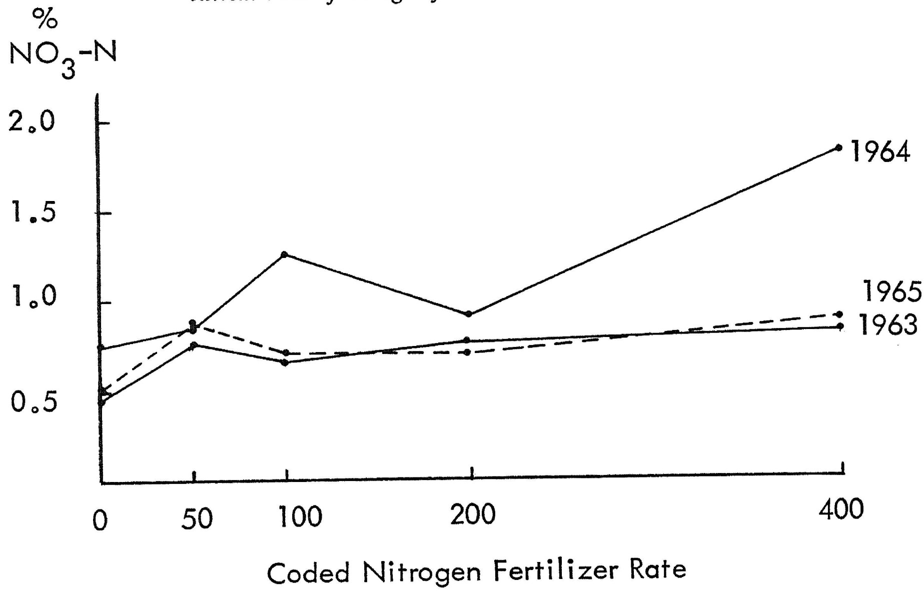


Figure 2. Percentages of nitrate nitrogen in Scarlet Globe red radish with various rates of nitrogen fertilizer.



Three red radish varieties were used in 1963 and the radishes were sampled at two different times about one week apart. Table 7 gives the analysis of variance of the nitrate nitrogen contents of the entire 1963 experiment. Fertilizer treatments and date of sampling both had significant effects upon the nitrate content of the three varieties. The lack of significant differences between varieties is interesting in light of the results over the three years as presented above where there was a significant varietal difference.

The mean nitrate nitrogen contents of 0.76 and 1.05 percent were obtained May 12 and May 18, 1963, respectively. This observation is contrary to the expected results of decreasing nitrate content with increasing maturity. There is no clear explanation for this anomaly. The radishes at both dates were within the size limits of radishes sold in grocery stores, although the mean diameter of the radishes sampled May 12 was smaller than that of the ones sampled May 18.

No interactions were significant, but the treatment means for each date, as well as the overall means, are given in Table 8 for interest. The 0 and 50 treatments resulted in significantly lower nitrate contents than the other treatments. There were no other significant treatment effects and the only trend seemed to be a slightly lower nitrate content in radishes grown where micronutrients were added to the base treatment.

In most plants the reduction of nitrate to amino nitrogen compounds probably takes place in the leaves where the amino nitrogen is chemically combined interaction which necessitated the separation of the data by variety to determine if any true differences in nitrate content existed between the plant parts (Table 9.) The two varieties accumulated different nitrate quantities, although the part-by-variety interaction detracts somewhat from this result. The overall means for the tops were 0.95 and 0.84 percent NO_3-N and for the roots, 0.82 and O.76 percent for Champion and Scarlet Globe, respectively, (Figure 3).

Table 10 gives results of the analyses of variance of the nitrate nitrogen contents of both tops and roots of the two varieties. This table indicates that the part-by-variety interaction shown in Table 9 arose from the large difference between nitrate contained in the roots and tops of the Champion variety, whereas there was no difference in nitrate content between the two parts of the Scarlet Globe variety. (See Figure 3).

TABLE 9--ANALYSIS OF VARIANCE OF THE NITRATE NITROGEN CONTENTS OF RED RADISHES GROWN IN 1965

Source	df	ms	Level of Significance*
Replicate	2	.2202	-
Treatment (Tmt)	8	.3557	1%
Error a	16	.0309	-
Variety (Var)	1	.5194	5%
Var x Tmt	8	.0583	NS
Error b	18	.0744	-
Part	1	.0806	NS
Part x Variety	1	.1883	1%
Part x Tmt	8	.0368	NS
Part x Var x Tmt	8	.0312	NS
Error C	36	.0238	-
Total	107	-	-

*The letters ns indicate lack of significant effects.

TABLE 10--ANALYSIS OF VARIANCE OF THE 1965 RED RADISH NITRATE NITROGEN CONTENTS BY VARIETY

		Variety			
		Scarlet Globe		Champion	
Source	df	m.s.	Level of significance	m.s.	Level of significance
Replicate	2	.2892	-	.1298	-
Treatment	8	.2676	1%	.1464	5%
Error A	16	.0513	-	.0385	-
Part	1	.0112	NS	.2585	1%
Part x Tmt	8	.0352	NS	.0328	NS
Error B	18	.0206	-	.0270	-
Total	53	-	-	-	-

TABLE 7--ANALYSIS OF VARIANCE OF THE NITRATE NITROGEN CONTENTS OF THREE VARIETIES OF RED RADISHES SAMPLED ON TWO DATES IN 1963

Source	df	m.s.	Level of Significance*
Replicate	2	0.7705	-
Variety (V)	2	0.6244	N.S.
Error A	4	0.1048	-
Treatment (Tmt)	8	0.2342	1%
Error B	16	0.0588	-
V x Tmt	16	0.0358	N.S.
Error C	32	0.0302	-
Date	1	3.1000	5%
Error D	2	0.0575	-
Date x V.	2	0.1048	N.S.
Error E	4	0.2705	-
Date x Tmt	8	0.0313	N.S.
Error F	16	0.0378	-
Date x V x Tmt	16	0.0207	N.S.
Error G	32	0.0343	-
Total	161	-	-

*N.S. indicates no significant differences.

TABLE 8--MEAN NITRATE NITROGEN CONTENTS OF RED RADISHES GROWN UNDER DIFFERENT FERTILITY TREATMENTS AND HARVESTED AT TWO DIFFERENT DATES IN 1963

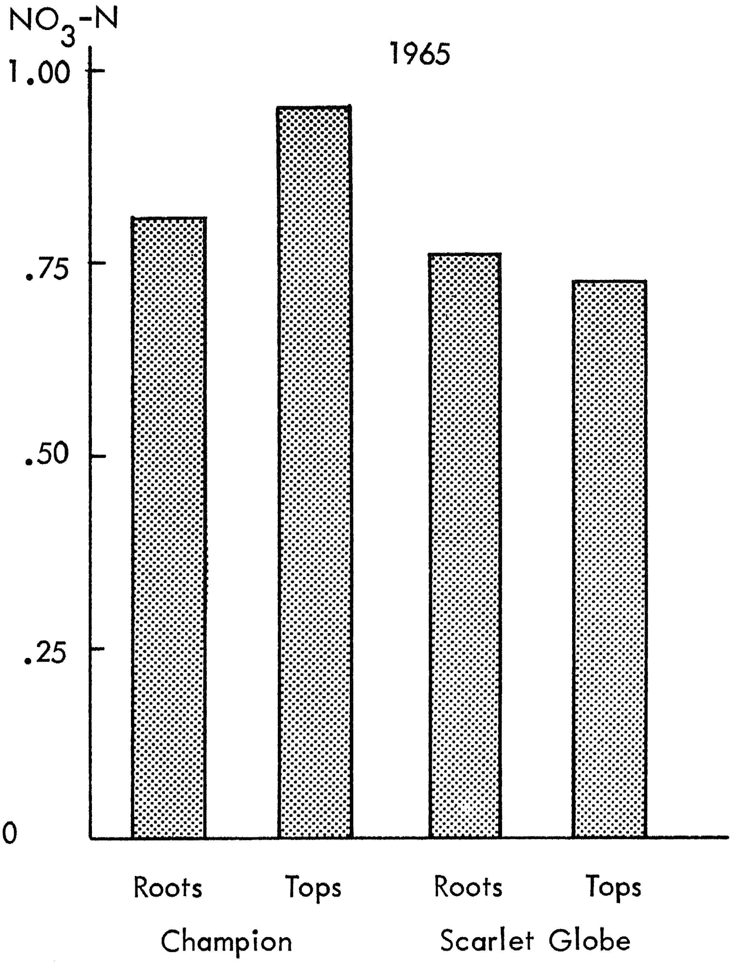
Treatment*	Date: May 12	Date: May 18	Overall Mean+
	% NO_3-N		
0	0.53	0.87	0.70[a]
50	0.71	0.89	0.80[a]
100	0.69	0.99	0.84[b]
200	0.76	1.18	0.97[b]
400	0.81	1.19	1.00[b]
$200P_0$	0.87	1.13	1.00[b]
$200K_0$	0.87	1.16	1.02[b]
$200L_0$	0.85	1.08	0.96[b]
200T	0.72	0.95	0.84[b]

*Treatment codes are explained in Table 2.

+Means with the same superscript are not significantly different at the 5% level.

with compounds formed during or subsequent to photosynthesis. During the initial stages of this experiment the question of the effect of treatment upon nitrates in the leaves of radishes arose. In 1965 radish leaf samples were taken at the same time as the root samples. The analysis of variance of the combined data obtained in 1965 showed that there was a significant part of plant-by-variety

Figure 3. Percentages of nitrate nitrogen in roots and tops of two radish varieties.



The nitrate data for each plant part grown in 1965 were analyzed further statistically; no treatment effect was shown to be significant due to a marked differential response in nitrate accumulation by the radish tops (Tables 9 and 10). The roots showed no significant response to treatment, although there was a tendency for the nitrate content of radish roots grown on the 0 treatment to be less than the other treatments and for the nitrate content of radishes grown on the 400 treatment to be highest in nitrate.

Table 11, which gives the mean nitrate content for each part of each variety by treatments, illustrates these points.

The mean nitrate levels in the tops show a curious tendency in response to increasing nitrogen fertilizer. Two maxima are illustrated with one at the 50 treatment and the other at the 400 treatment. This effect of nitrogen fertilizer was discussed in the early report on this experiment (4). The additional years of data have failed to give any better understanding of this phenomena. Table 11 shows that the effect of the alterations in the basic treatment had mixed and inconsistent effects on the nitrate contents of the red radish plant in 1965.

Overall, the data indicate an increase in nitrate nitrogen content with increasing rates of nitrogen fertilizer in red radishes. No consistent influence was shown on the nitrate content of the radishes when elements were omitted from the basic fertilizer mixture. The untreated soil was deficient in phosphorus, as observed from rate and quantity of growth, but this obvious phosphorus deficiency had little effect on the nitrate content of the radishes, except perhaps by causing a slight reduction in nitrate accumulation on the $200P_0$ treatment.

While the overall analysis revealed no variety-by-year interaction, the 1965 data showed that in this one year the Champion variety tended to contain more nitrate on a given treatment than did the Scarlet Globe variety. When the data for the three years was pooled this varietal difference was still significant. The effect of parasitic infestation on increased nitrate accumulation was evident in 1964.

White Radish

White radishes, commonly called icicle radishes, are frequently grown in spring gardens. Actually, "icicle" refers to a specific variety which, coincidently, was used in this study. The white radish has a longer root and requires a few more days to reach maturity than its red counterpart.

In this study, the aphid problem encountered in 1964 on the red varieties also occurred on the white variety. The statistical analysis showed a significant effect of year but this effect was associated with the insect problem. The 1964 data showed a mean nitrate nitrogen percentage of 2.31, compared to 1.16 and 1.30 percent in 1963 and 1965, respectively. There was no statistically significant difference between the nitrate contents of the icicle radishes in 1963 and 1965. Because of the insect problem associated with the 1964 crop of white radishes, only the data from the 1963 and 1965 crops will be considered in the following discussion.

Statistical analysis of the 1963 nitrate data showed no significant differences between treatments. Great variation occurred between the nitrate contents of radishes from plots that were treated alike, as pointed out in the preliminary report (4). Due to this variation a very striking treatment effect would be required to achieve significance.

In 1965 roots and tops were sampled at the same time, but no significant differences were observed in nitrate contents of the two plant parts. The effect of treatments, however, was highly significant. The test of the means by the New Duncan's Multiple Range Test showed that the nitrate content of the radishes grown under the highest nitrogen level was significantly greater than the nitrate

TABLE 11--MEAN NITRATE NITROGEN CONTENTS OF RED RADISH ROOTS AND TOPS GROWN UNDER DIFFERENT FERTILITY TREATMENTS IN 1965

Variety	Part	Treatments*								
		0	50	100	200	400	$200P_0$	$200K_0$	$200L_0$	200T
Champion	Root	.59	.83	.81	.73	1.11	.80	.86	.77	.86
Scarlet Globe	Root	.48	.83	.70	.69	.88	.56	.88	.96	.88
Champion	Top	.61	1.15	.71	1.04	1.25	1.01	.85	1.01	.96
Scarlet Globe	Top	.26	.71	.64	.56	1.17	.60	.88	1.04	.74

*Treatments are coded according to Table 2.

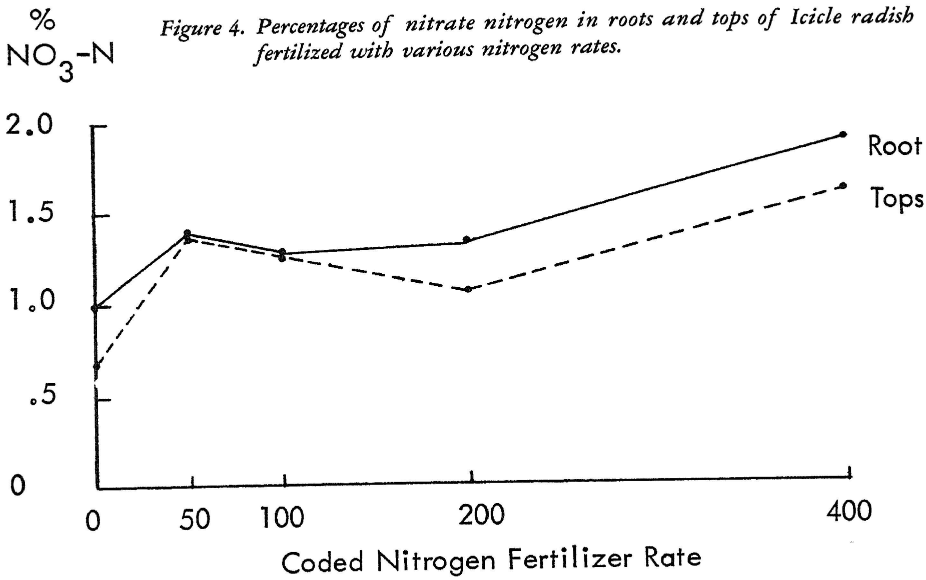


Figure 4. Percentages of nitrate nitrogen in roots and tops of Icicle radish fertilized with various nitrogen rates.

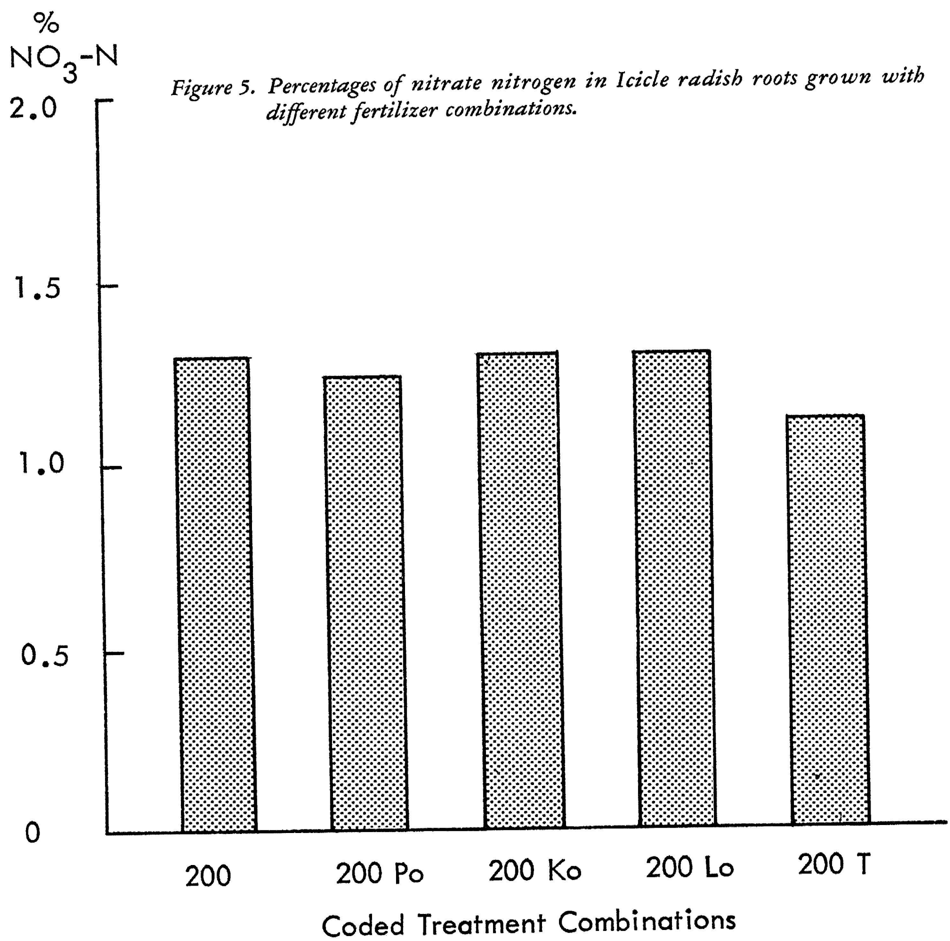


Figure 5. Percentages of nitrate nitrogen in Icicle radish roots grown with different fertilizer combinations.

content of the radishes grown under the other treatments. There was no statistically significant difference in nitrate content of the radishes grown under the other treatments. Figures 4 and 5 show the effects of the treatments upon the Icicle radish nitrate contents.

Beets

Table or red beets are commonly grown in home gardens as well as on truck farms. This species matures later than radishes. When the nitrate contents of beet roots were analyzed statistically, several significant interactions were found between varieties, treatments, and years. After studying these responses it became obvious that year-to-year consideration of the data was required. As stated in a previous report (4), no significant response of beets to treatment occurred in the 1963 tests, so this discussion will concern only the 1964 and 1965 data (4).

In 1964 both roots and tops were sampled on the 26th of June and an additional root sample was taken on July 19. The statistical analysis of the plant part sampling of June 26 showed significant differences due to treatment and to plant part with no significant interactions or variety difference in terms of nitrate content. The effect of treatment was most pronounced on the tops as shown in Figures 6 and 7. According to the Duncan Multiple Range Test only the highest nitrogen treatment was significantly different from the other treatments.

Date of harvest of the roots had a significant effect on the nitrate content. The roots harvested July 19 were consistently higher in nitrate than were the early sampled roots. Increasing rates of nitrogen fertilization caused an increase in nitrate accumulation in the beet roots sampled at the early date. The only

Figure 6. Percentages of nitrate nitrogen in roots and tops of red table beets fertilized with various rates of nitrogen.

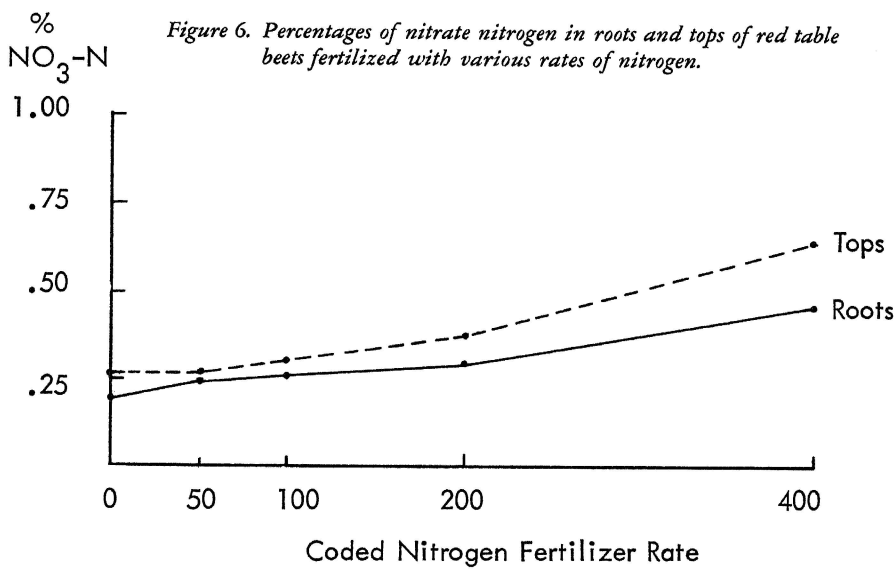
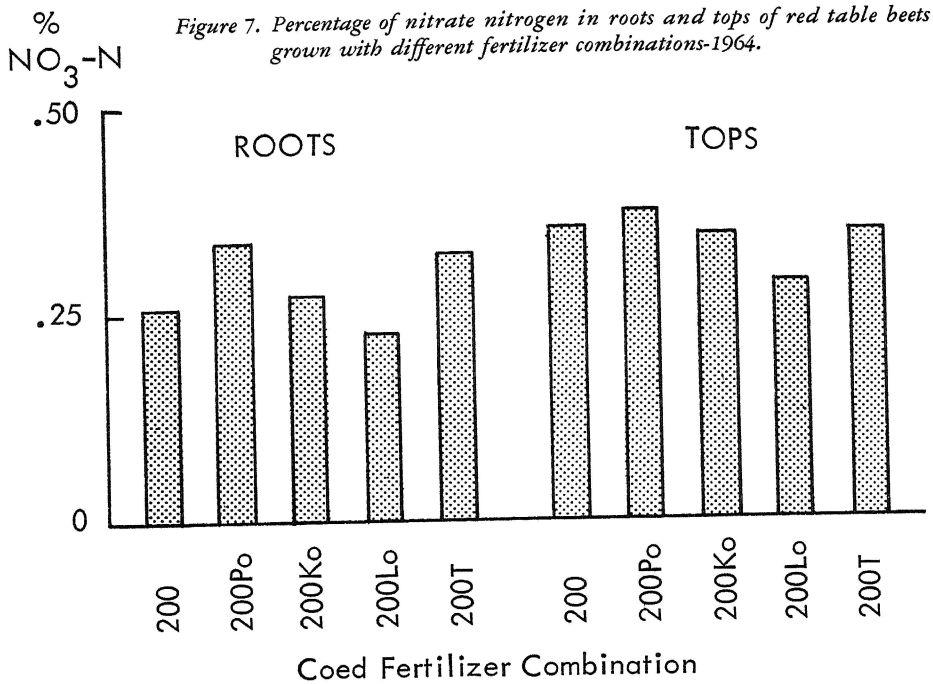


Figure 7. Percentage of nitrate nitrogen in roots and tops of red table beets grown with different fertilizer combinations-1964.



indication of a difference in response to fertilizer on the two dates was that the presence of trace elements in the basic treatment appeared to lower nitrate accumulation at the late sampling date (Figures 8 and 9). As discussed in the section on radishes the spring of 1964 was cool and wet. The increase in nitrate in the beets at the late harvest may reflect increased mineralization of nitrogen in response to warmer soil temperatures.

In 1965 the tops and roots of the table beets were sampled as in 1964. The analysis of the nitrate content data (Table 12), showed that the main effects of treatment and plant part were significant. The significant interaction involving plant part necessitated separation of the data on the plant parts for further analysis. When separated, the data revealed the fertilizer treatments had no effect on the nitrate content of the roots but they had a highly significant effect on the nitrate content of the tops.

A Duncan Multiple Range Test was performed on the mean nitrate content of the tops (Table 13). The omission of potassium or lime from the basic treatment caused an increase in nitrate content in the beet tops. The curious response to increasing nitrogen rates noted with radish leaves also occurred with beet tops, although the effect was not large enough to be significant at the 5 percent level.

Figure 8. Percentages of nitrate in red table beet roots fertilized with various rates of nitrogen sampled twice-1964.

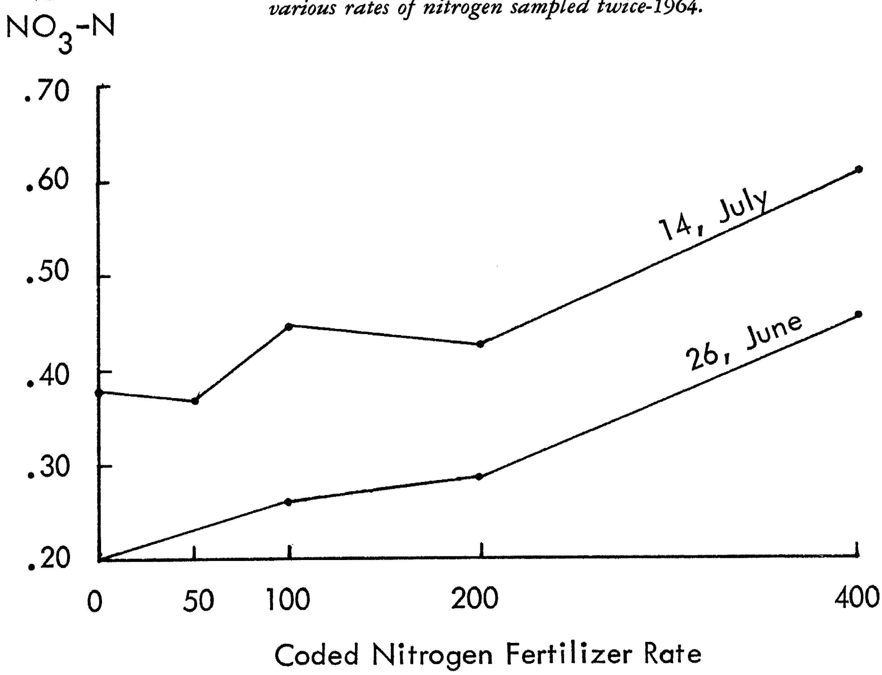


Figure 9. Percentage of nitrate nitrogen in red table beets grown under different fertilizer combinations and sampled at different dates.

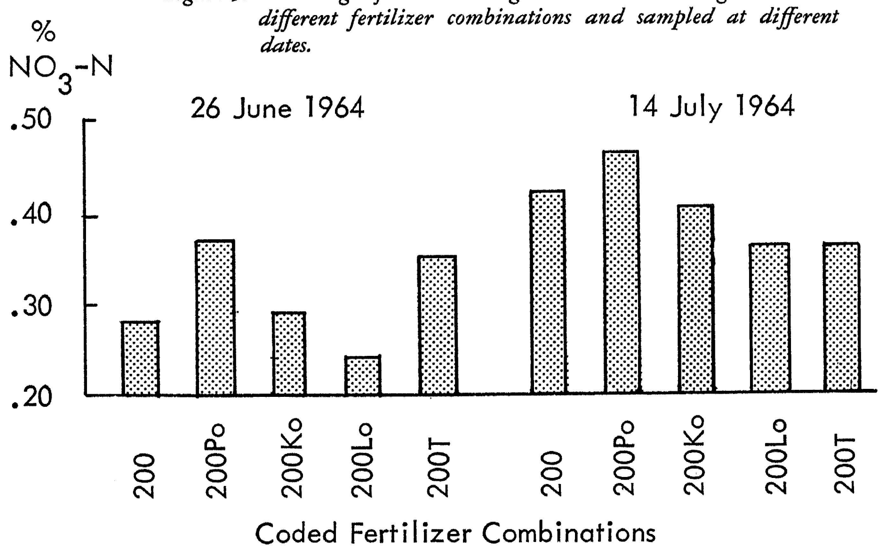


TABLE 12--ANALYSIS OF VARIANCE OF NITRATE CONTENT OF TABLE BEETS AND ROOTS SAMPLED IN 1965

Source	df	m.s.	Significance
Replicate	2	.0686	-
Treatment	8	.1951	1%
Error a	16	.0456	-
Variety	1	.0502	NS
Var x Tmt	8	.0225	NS
Error b	18	.0359	-
Part	1	1.8696	1%
Part x Var	1	.0829	5%
Part x Tmt	8	.0953	1%
Part x Var x Tmt	8	.0341	NS
Error c	36	.0302	-
Total	107	-	-

TABLE 13--MEAN NITRATE NITROGEN CONTENTS OF BEET LEAVES - 1965

	Treatments*								
	0	50	100	200	400	$200P_O$	$200K_O$	$200L_O$	200T
%NO_3-N	.19	.58	.42	.67	.78	.50	.82	.85	.61
Duncan's + Significance	d	bc	cd	abc	ab	c	ab	a	abc

*Treatments are coded according to Table 2.

+All treatments which are over the same letter were not significantly different according to Duncan's Multiple Range test.

The data indicate that nitrate accumulation in beet roots is not affected to any large extent by nitrogen fertilization. On the other hand the rate of nitrogen applied to the plots did affect the nitrate content of beet tops. The effect of varying the composition of the basic fertility treatment on the nitrate content of beet roots over the three years of the study was inconsistent. The results of the date of sampling study made on the beet tops in 1964 was similar to the results obtained in 1963 on radishes in that nitrates tended to increase at the later sampling date.

Turnips

Turnips are grown in home gardens for both greens and roots. In fact, there are varieties available which have been bred for leaf production as well as varieties used primarily for roots or for both leaves and roots. Included in this study was one variety, Shogoin, of the leaf type and one universal variety, Purple Top White Globe. Both varieties were sampled for greens. Since the Shogoin did not develop a root of commercial value, only the Purple Top White Globe roots were sampled.

Table 14, which contains the analysis of variance of the nitrate contents of turnip tops for the years 1963 and 1965, shows that year did not significantly influence the nitrate content of turnip tops. However, all interactions involving

TABLE 14--ANALYSIS OF VARIANCE OF NITRATE NITROGEN IN TURNIP TOPS IN 1963 AND 1965

Source	df	m.s.	Level of Significance
Replicate	2	.0173	-
Year	1	.6364	NS
Error A	2	.6364	-
Treatment (Tmt)	8	.6640	1%
Year x Tmt	8	.1488	5%
Error B	32	.0498	-
Variety (V)	1	.3997	1%
Year x V	1	2.1028	1%
V x Tmt	8	.0671	NS
V x Year x Tmt	8	.1068	1%
Error C	36	.0337	-
Total	107	-	-

year were significant, which indicates that response, as measured by nitrate content, to variety or treatment was not the same in the two years included in this analysis. (The data from 1964 were not included due to insect- and weather-induced stand and growth problems). The significant interactions necessitate separate consideration of the data obtained in each year.

The 1963 data were covered in an earlier report; only a brief summary will be given here for that year (4). The nitrate contents of the turnip tops grown

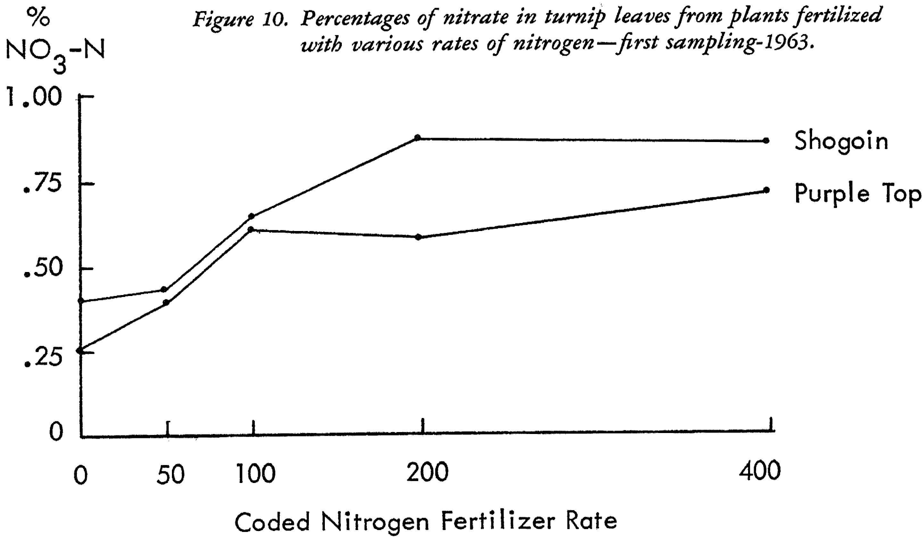


Figure 10. Percentages of nitrate in turnip leaves from plants fertilized with various rates of nitrogen—first sampling-1963.

in 1963 were affected significantly by nitrogen treatment but not by the various combinations of nutrients. Figure 10 illustrates the effect of nitrogen fertilizer upon the nitrate content of the turnip leaves grown in 1963 (Figure 10 covers only the first harvest. The effect of harvest date will be discussed later).

Table 15, which contains the analysis of variance of the 1965 turnip leaf data, indicates significant differences in nitrate contents due to both treatment and varieties. In addition, the two varieties did not respond in the same fashion

TABLE 15--ANALYSIS OF VARIANCE OF THE NITRATE CONTENT IN TURNIP LEAVES, 1965

Source	df	m.s.	Level of Significance
Replicate	2	.3516	-
Treatment (Tmt)	8	.6024	1%
Error a	16	.0510	-
Variety (V)	1	2.1680	1%
V x Tmt	8	.1625	5%
Error b	18	.0505	-
Total	53	-	-

to the treatments as shown in Figures 11 and 12. Because of the significant interactions, the means for each treatment were calculated for each variety and tested by Duncan's Multiple Range Test (Table 16). From these tests it can be concluded that significantly higher nitrate levels were found in the turnip leaves at the 400 nitrogen level and significantly lower nitrate levels were found at the 0 nitrogen level. There were minor differences in response to the 50, 100, and

Figure 11. Percentage of nitrate nitrogen in turnip tops from plants fertilized with various rates of nitrogen-1965.

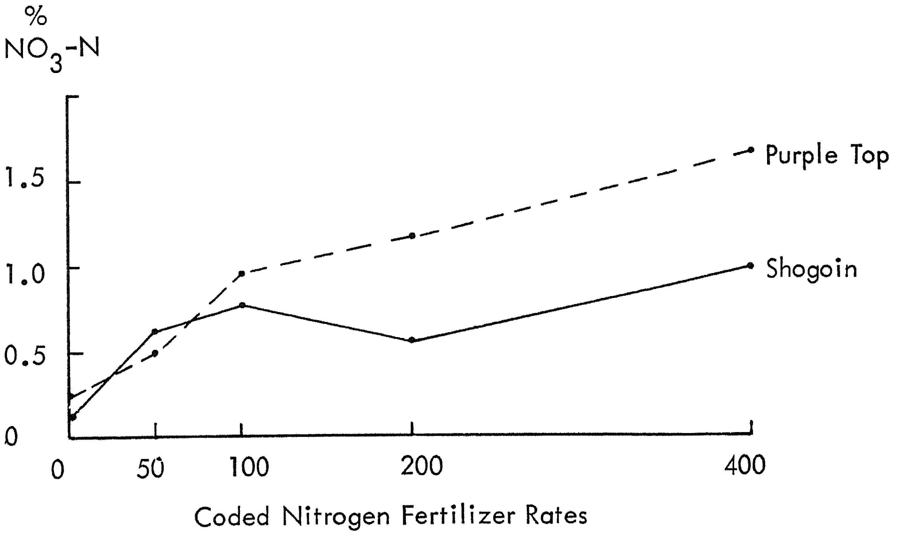


Figure 12. Percentage of nitrate nitrogen in turnip tops from plants grown with different fertilizer combinations-1965.

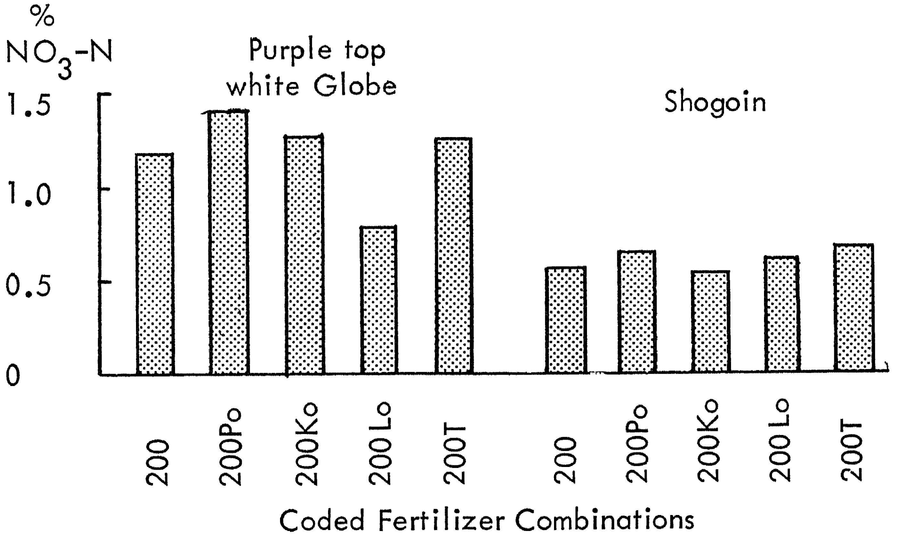


TABLE 16--MEAN NITRATE NITROGEN CONTENTS OF TWO VARIETIES OF TURNIP LEAVES GROWN IN 1965

	Treatment*								
Variety	0	50	100	200	400	200P$_0$	200K$_0$	200L$_0$	200T
Purple top+	.26^{a}	.49^{ae}	.95^{cde}	1.17^{cd}	1.68^{b}	1.37^{bc}	1.24^{bc}	.74de	1.23^{bc}
Shogoin+	.12^{a}	.63^{c}	.79^{bc}	.56^{c}	.98^{b}	.65^{bc}	.51^{c}	.61^{c}	.66^{bc}

*Treatments coded according to Table 2.

+Within a given variety means with the same superscript are not significantly different according to Duncan's Multiple Range Test.

200 nitrogen levels between varieties although there was an overall tendency for nitrates in the leaves to increase with increasing rates of nitrogen fertilizer. The alteration of the basic fertility treatment by omission did not give a significant effect on nitrates in the Shogoin leaves, but omission of lime significantly reduced the nitrate content of Purple Top White Globe leaves.

A comparison of results from the 1963 and 1965 portions of this study showed that in 1963 the Shogoin tops had higher nitrate contents than the Purple Top White Globe tops but this standing was reversed in 1965. Due to the many factors that affect the nitrate accummulation in plants it is impossible to explain this marked difference between years on the two varieties.

The 1964 turnip root data was inconclusive and will not be discussed. In the preliminary report it was shown that a significant response to treatment was obtained in 1963 (4). In addition, a significant date-of-harvest effect was obtained with a lower nitrate content at the last sampling.

In 1965 no significant treatment effect on nitrate content of turnip roots was obtained. A study of the nitrate data from 1965 showed there was a significant response to nitrogen whereas the results from the fertilizer balance portion of the study showed inconsistent response to the four treatments. From the statistical standpoint, bias is introduced by selecting a portion of a study for separate analysis but this course of action was taken here because of the obvious treatment effects.

The nitrogen portion of the study was analyzed separately with the results shown in Table 17. The nitrate contents of the turnips were similar in magnitude to those obtained in 1963, although a more marked response to nitrogen

TABLE 17--STATISTICAL ANALYSIS OF NITRATE CONTENTS OF PURPLE TOP WHITE GLOBE TURNIP ROOTS, 1965
(Nitrogen rate portion of the study)

A. Analysis of Variance

Source	df	m.s.	Level of Significance
Replicate	2	.0234	-
Treatment	4	.1620	5%
Error	8	.0352	-
Total	14	-	-

B. Duncan Multiple Range Test of Mean Nitrate Nitrogen

	Treatment				
	0	50	100	200	400
$\%NO_3$-N in roots	$.29^{b}$	$.32^{b}$	$.45^{ab}$	$.71^{a}$	$.80^{a}$

*Means with the same superscript are not significantly different.

was obtained in 1965. The greater response in 1965 may be attributed to residual soil levels of nitrogen from previous years. This tentative conclusion was reached because the nitrate content of turnips grown on the 0 nitrogen plots was lower in 1965 than in 1963, whereas the nitrate content of turnips grown on the 400 nitrogen treatment was higher in 1965 than in 1963. In both years, increased nitrogen application caused greater nitrate levels in the turnips.

Carrots

A poor stand of carrots was obtained in 1965 but the data that were obtained indicated responses similar to those obtained in 1963 and 1964. The mean nitrate contents by treatment and year for the two carrot varieties are given in Table 18. On 1964 there was a significant difference between varieties with the Nantes variety being the highest in nitrate. From the practical standpoint, however, the magnitude of the nitrate levels in the carrot roots indicates that this species did not accumulate appreciable nitrate under the conditions of the experiment.

TABLE 18--MEAN NITRATE NITROGEN CONTENT OF CARROT ROOTS 1963 AND 1964

Year	Variety	Treatment*								
		0	50	100	200	400	$200P_o$	$200K_o$	$200L_o$	200T
						% NO_3-N				
1963	Red Core	.02	.02	.02	.04	.03	.03	.02	.05	.03
1963	Nantes	.03	.02	.03	.05	.05	.04	.06	.07	.06
1964	Red Core	.02	.02	.02	.03	.04	.04	.05	.05	.03
1964	Nantes	.03	.04	.05	.06	.05	.09	.05	.03	.06

*See Table 2 for the explanation of the treatment codes.

Leaf Lettuce

As mentioned previously, nitrates most frequently are expected to accumulate in the leaves of plants. Leaf lettuce is a common garden crop and the leaves of this crop are consumed raw; therefore, there would be no possible loss of nitrate in the process of cooking. With cooked vegetables, such as turnip greens, there is a possibility that nitrates would be partially leached out by boiling water..

The analysis of variance of the nitrate content of leaf lettuce grown in the three years of this study (Table 19) shows that fertilizer treatments significantly

TABLE 19--ANALYSIS OF VARIANCE OF THE NITRATE NITROGEN CONTENT OF LEAF LETTUCE GROWN IN THREE DIFFERENT YEARS

Source	df	m.s.	Level of Significance
Year	2	.0945	NS
Replicate in Year	6	.1422	5%
Treatment (Tmt)	8	.2534	5%
Error A	64	.0336	-
Variety (V)	1	.0010	NS
V x Tmt	8	.0196	NS
V x Year	2	.0050	NS
V x Tmt x Year	16	.0131	NS
Error B	54	.0118	-
Total	161	-	-

influenced the nitrate content of the lettuce. The mean nitrate contents from the nine treatments are given in Table 20 with the results of the new Ducan Multiple Range test of significance. The 400 nitrogen rate resulted in significantly higher nitrate content in the lettuce than all other treatments. The $200P_0$ treatment appears to have caused lower nitrate levels than other treatments. In 1965, the $200P_0$ plots did not have sufficient growth for sampling. For statistical purposes these plots were counted as having zero nitrate, thus introducing some bias into the means given in Table 20. The 0 nitrogen treatment resulted in significantly less nitrate in the lettuce than all other treatments.

The results demonstrate that if the fertility treatments were well balanced there was no great effect on nitrate in lettuce. The significance of the results in terms of human response awaits definition by the medical profession of a critical level for consumption of nitrate.

A detailed date of harvest study was made on the lettuce grown in the spring of 1964. The plots were sampled on three dates, June 3, 16, and 24. The analysis of variance given in Table 21 shows a highly significant date-by-treatment interaction and a treatment effect significant at the 5 percent level of probability. Figures 13 and 14 demonstrate the significant effects.

TABLE 20--MEAN NITRATE NITROGEN CONTENT OF LEAF LETTUCE (1963, 1964, and 1965)

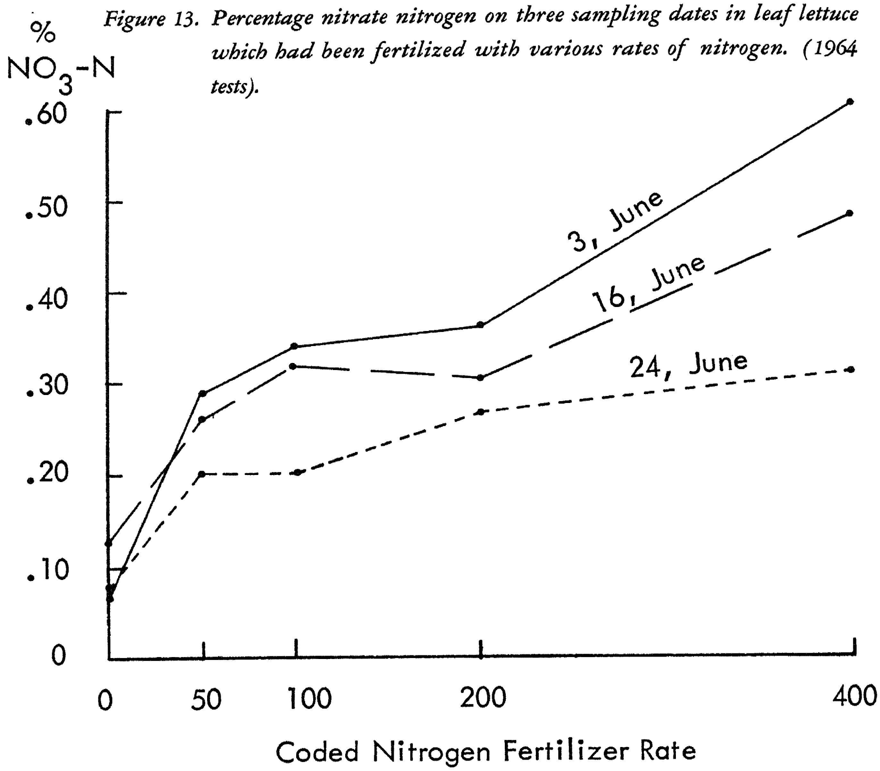
	Treatment								
	0	50	100	200	400	$200P_o$	$200K_o$	$200L_o$	200T
$\%NO_3$-N*	$.08^{f}$	$.25^{de}$	$.27^{de}$	$.25^{de}$	$.49^{a}$	$.22^{e}$	$.35^{bc}$	$.41^{b}$	$.32^{cd}$

*According to Duncan's Multiple Range Test any means with the same superscript are not significantly different at the 5% level.

TABLE 21--ANALYSIS OF VARIANCE OF THE NITRATE NITROGEN CONTENT OF LEAF LETTUCE GROWN IN SPRING, 1964

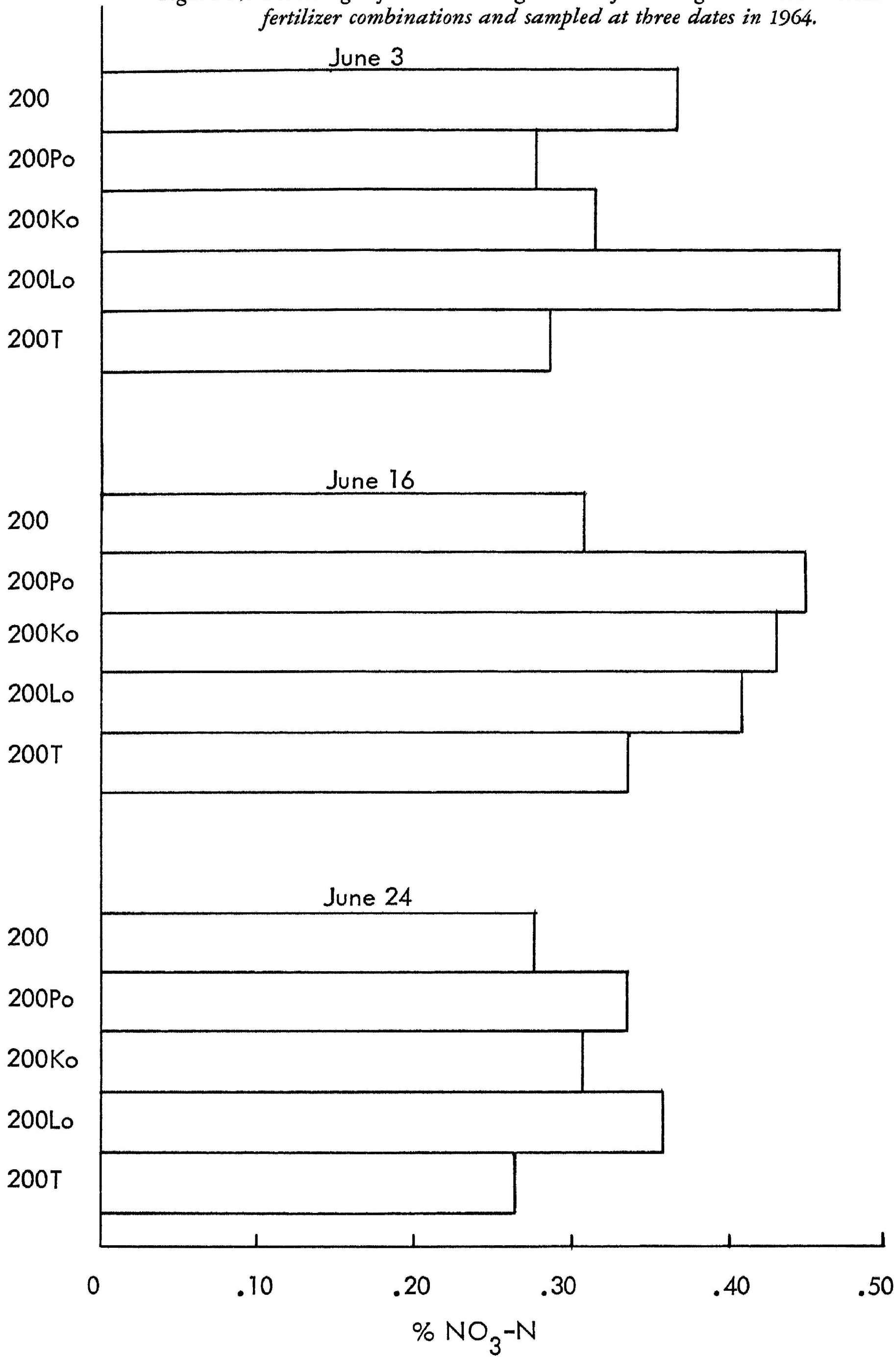
Source	df	m.s.	Level of Significance
Replicate	2	.7389	-
Treatment	8	.1912	5%
Error A	16	.0601	-
Variety	1	.0071	NS
Var x Tmt	8	.0068	NS
Error B	18	.0132	-
Date	2	.1230	1%
Date x Var	2	.0026	NS
Date x Tmt	16	.0211	1%
Date x Tmt x Var	16	.0113	NS
Error C	72	.0087	-
Total	161	-	-

Figure 13. Percentage nitrate nitrogen on three sampling dates in leaf lettuce which had been fertilized with various rates of nitrogen. (1964 tests).



Nitrate content of lettuce decreased with increasing maturity except when grown on the 0 nitrogen, $200P_0$, $200K_0$, and 200T treatments, thus demonstrating the fact that response to some treatments changed with maturity of the lettuce. The effect of nitrogen rate shown in Figure 13 is a typical nitrogen response. The omission of lime from the basic treatment appeared to increase the nitrate content all three dates. Since liming increases molybdenum availability it might be reasonable to assume that the plots had a marginal molybdenum level for lettuce when unlimed. Molybdenum is required in the plant for nitrate reduction, hence the increase in nitrate contents in the leaf lettuce from the unlimed plots. When the radishes, beets, and turnips which were discussed above are considered, molybdenum availability as an explanation of the increased nitrate in leaf lettuce grown on the unlimed plots becomes untenable unless lettuce has a higher molybdenum requirement than the other plants. The conclusion must be that the response in the absence of lime cannot be fully explained with the data available.

Figure 14. Percentage of nitrate nitrogen in leaf lettuce grown with various fertilizer combinations and sampled at three dates in 1964.



Semi-Head Lettuce

The initial plans of the experiment called for two varieties of semi-head lettuce to be used to study nitrate accumulation. In 1963 no significant differences were observed between treatments or varieties in respect to nitrate accumulation. One variety, Buttercrunch, was dropped from the study based upon the lack of varietal differences in 1963.

The Bibb variety gave no response to any treatment in 1964. In 1965 the Bibb variety was planted but no satisfactory heads developed due, at least in part, to hot weather.

Although the lack of response to treatment in 1963 and 1964 should be recognized, the mean nitrate contents of the heads grown (Table 22) demonstrate a tendency for the dual nitrate maxima to occur in response to rate increases of nitrogen fertilizer. Other than this tendency to display the unusual response to nitrogen, the only other conclusion that may be made is that the 0 nitrogen treatment tended to result in low levels of nitrate in the lettuce.

Mustard

Mustard is a species of vegetable commonly grown for greens. Two varieties were grown in 1963 and three in 1965. Mustards grown in 1964 were not sampled because of the combined effects of insects and wet weather which caused poor germination and growth. Table 23 gives the overall means of the nitrate content of mustard grown in 1963 and 1965. There were no significant differences between varieties, therefore, pooling of the data is justified.

The effect of nitrogen treatment was one of increasing nitrate content with the increasing rates of nitrogen fertilizer. In 1965, the no nitrogen treatment resulted in significantly less nitrate than all other treatments and the highest nitrogen treatment resulted in the greatest nitrate accumulation. These effects were also present in 1963 but were not so obvious. No significant effects of the remaining treatments on nitrate accumulation were found, although the omission of phosphorus from the basic treatment tended to result in lower nitrate content both years. The effect of phosphorus omission may have resulted in reduced root proliferation and, consequently, limited the root system, resulting in less nitrate uptake by the plant.

Kale

As pointed out in the preliminary report, kale exhibited a strong and unique response to increasing rates of nitrogen fertilizer. That response consisted of an initial nitrate content obtained at 50 pounds nitrogen rate followed by a minimum with increased nitrogen rates and a second maximum at the 400 pound rate (4). In subsequent years this observation tended to prevail but not to the marked degree found in 1963. The 1964 spring crop did not contribute sufficient data since it suffered a fate similar to other vegetables grown that year: Poor stands of kale were obtained in the spring due to the cold, wet growing period

TABLE 22--MEAN NITRATE NITROGEN CONTENTS OF SEMI-HEAD LETTUCE GROWN IN 1963 AND 1964
(% Nitrate nitrogen - dry matter basis)

Year	Variety	Treatment*								
		0	50	100	200	400	$200P_0$	$200K_0$	$200L_0$	200T
1963	Bibb	.13	.41	.45	.42	.48	.35	.39	.52	.42
1963	Butter Crunch	.19	.39	.24	.31	.36	.36	.26	.26	.42
1964	Bibb	.15	.32	.21	.22	.26	.26	.29	.23	.20

*Coded as described in Table 2.

TABLE 23--MEAN NITRATE NITROGEN CONTENTS OF MUSTARD LEAVES 1963 AND 1965

Year	Treatment								
	0	50	100	200	400	$200P_0$	$200K_0$	$200L_0$	200T
					% NO_3-N				
1963*	$.46^{d}$	$.64^{bcd}$	$.76^{abc}$	$.85^{ab}$	$.98^{a}$	$.81^{ab}$	$.87^{a}$	$.97^{a}$	$.95^{a}$
1965*	$.42^{c}$	$.82^{b}$	$.87^{b}$	$.87^{b}$	1.21^{a}	$.70^{b}$	$.92^{b}$	$.89^{b}$	$.95^{b}$

*Within a given year means with the same superscript are not significantly different.

which reduced germination and limited growth, especially on low phosphorus plots, to the extent that representative samples could not be obtained. In 1965 excellent kale growth was obtained and the twin-nitrate maximums were present, although ths effect was not statistically significant.

In 1965, an attempt was made to obtain a measure of the diurnal change in nitrate content. Samples were taken on the morning of June 25, and additional ones in the afternoon. There was no significant difference between the samples, although there was a tendency for the nitrate content of the kale to be slightly lower in the afternoon.

Table 24 gives the means obtained from the kale portion of the study. There was a significant difference between varieties in 1965. (Only one variety was used in 1963.)

Spinach

Germination problems were encountered with spinach in 1963 and 1964. This problem resulted in a complete crop failure in 1963 and many missing plots in 1964, which precluded statistical analysis of the data. The means from the 1964 crop are included in Table 25 for informational purposes only.

An excellent spinach crop was obtained in 1965, except that growth on the plots receiving no phosphorus was much less than that on all other plots. Two varieties of spinach were grown in 1965, but there was no difference in nitrate accumulation between the varieties; therefore, the data were pooled to obtain the mean for 1965 given in Table 25. The effect of treatment was highly significant so the individual means were tested by the Duncan Multiple Range Test with the results shown in Table 25.

The test of the mean nitrate contents obtained in 1965 showed that the rate of nitrogen had a significant effect upon the nitrate content of the spinach. Increasing the rate of nitrogen fertilizer resulted in increased nitrate content. While indications were that the omission of nutrients from the basic treatment had no significant effect on nitrate content of the spinach, there was a tendency for all treatments which did not include a specific nutrient to contain higher levels of nitrate than the full basic treatment (Treatment 200).

Swiss Chard

Swiss chard was added to the experimental plan in 1964 and 1965. The chard was sampled two different times in 1964, but there was no significant effect of date of sampling upon nitrate content. In 1965 only one sample was taken. The effect of treatment was significant at the 5 percent level in 1964, but was not statistically significant in 1965. The means for both years are in Table 26, also, the results of the Duncan's Multiple Range test on the 1964 data.

Consideration of the 1965 data given in Table 26 indicates a treatment effect. The raw data showed a large intratreatment variation which was not consistent with replicate, resulting in an unusually high standard deviation of the means;

TABLE 24--MEAN NITRATE NITROGEN CONTENT OF KALE GROWN IN 1963 AND 1965

Year	Variety	Treatment								
		0	50	100	200	400	$200P_0$	$200K_0$	$200L_0$	200T
		% NO_3-N								
1963	Siberian Improved	.30	.69	.48	.34	1.02	.60	.61	.52	.38
1965	Siberian Improved*	.41	.49	.36	.57	.65	.50	.54	.53	.50
1965	Dwarf Blue Schotch*	.52	.49	.64	.72	.94	.87	.76	.98	.72

*Significant differences between varieties in 1965

TABLE 25--EFFECT OF FERTILIZER TREATMENT UPON NITRATE CONTENT OF SPINACH IN 1964 AND 1965

Year	Treatment								
	0	50	100	200	400	$200P_0$	$200K_0$	$200L_0$	200T
	% NO_3-N								
1964	.09	.20	.17	.18	.24	.14	.24	.42	.21
1965*	.13[e]	.23[de]	.31[cd]	.36[bcd]	.68[a]	.50[b]	.45[bc]	.51[b]	.30[cd]

*Means with the same superscript are not significantly different at the 5% level.

TABLE 26--EFFECT OF FERTILIZER TREATMENT ON NITRATE CONTENT OF SWISS CHARD IN 1964 AND 1965

Year	Treatments								
	0	50	100	200	400	$200P_0$	$200K_0$	$200L_0$	200T
	% NO_3-N								
1964*	.05[b]	.16[b]	.27[ab]	.26[a]b	.36[a]	.38[a]	.32[a]	.28[a]	.19[a]
1965	.02	.06	.32	.38	.60	.37	.57	.49	.53

*Means with the same superscript are not significantly different at the 5% level.

this in turn, resulted in lack of significant treatment effect. When the 1965 data is considered with the 1964 means, a tendency is noted for the omission of lime or potassium from the basic treatment to result in an increased nitrate content over the full basic treatment (treatment 200). The lower rates of nitrogen resulted in significantly less nitrate in the samples than the higher nitrogen rates (compare the 0 and 50 rates with the 200 and 400 rates).

Snapbeans

The 1963 and 1964 plantings included only vegetables from which the vegetative portions were usually consumed. The accumulation of nitrate in reproductive portions of plants has seldom been reported; therefore, to explore the effect of the treatments used in this study upon nitrate accumulation in an edible seed crop, snapbeans were added to the study in 1965. No treatment effect was found; in fact, the maximum nitrate content found in the green pods was only 0.09 percent nitrate nitrogen on the dry matter basis.

A single replicate of snap beans had been planted in the fall of 1964 when the nitrate contents were found to be higher than those found in the spring of 1965; they were still less than 0.20 percent NO_3-N on most nitrogen treatments.

Fall 1964

In the fall of 1964, kale, red and white radishes, mustard, and leaf lettuce were planted on the plots used in the spring. No additional fertilizer was added.

In general, the results were disappointing. No significant treatment effects appeared, due principally to great plot-to-plot variation. The nitrogen carryover from the spring treatment seemed to result in a tendency for the nitrate level in the vegetable to increase with increasing nitrogen as indicated in Table 27. The effect of the omission of certain elements from the basic treatment had mixed effects with no trend being evident.

No insect problems were encountered in the fall of 1964, and the nitrate contents of the 1964 fall crop of radishes are similar to those obtained in the spring of 1963 and 1965 (Table 6, Figures 2 and 3). The nitrate contents of the other vegetables listed in Table 27 are likewise similar to the contents found in the spring crops except that the fall-grown leaf lettuce appears to be higher in nitrate content than the spring grown lettuce (Table 20, Figures 13 and 14).

Grocery Survey

A survey of the nitrate contents of produce sold in Columbia, Mo., grocery stores was made the latter half of 1964. Fresh produce and canned baby food were sampled. Table 28 summarizes this survey. There appeared to be no seasonal trend in changes in nitrate content within the time period of this limited survey. Neither was there any tendency for vegetables from any one state to be markedly higher or lower than the vegetables from any other state.

TABLE 27--MEAN NITRATE CONTENTS OF VEGETABLES GROWN IN FALL, 1964

Species	Variety	Treatments 0	50	100	200	400	$200P_0$	$200K_0$	$200L_0$	200T
						$\% NO_3$-N				
Kale	Siberian Improved	.51	.58	.86	.59	.95	.66	1.31	.48	.85
Radish	French Breakfast	1.08	1.27	1.12	1.10	1.16	1.14	1.16	1.14	1.16
Radish	Cherry Belle	.76	.85	.80	.87	.99	.74	.97	.97	.98
Radish	Icicle	.91	.99	1.20	1.18	1.12	1.73	1.16	1.14	1.08
Mustard	Tendergreen	.77	.85	1.05	.98	1.08	1.34	.99	.88	.88
Mustard	So. Giant Curled	.77	.94	1.19	1.26	1.12	.86	1.15	.88	1.40
Lettuce	Black Seeded Simpson	.40	.52	.56	.34	.60	.42	.86	.70	.40

The results of the survey, in general, support some observations made during the field trials. For one, radishes are notorious nitrate accumulators. Lettuce and turnips have variable nitrate contents. The survey indicated that celery and endive had ranges in nitrate content similar to those of lettuce and turnips. Most of the other vegetables were relatively low in nitrate content. It is necessary to emphasize that this was a very limited survey.

The baby food portion of the survey gives rise to some concern. From the data it appears that beets and spinach may have relatively high nitrate contents. The medical literature indicates that infants are quite sensitive to nitrates in the diet. In addition, it is seldom that an infant will consume at one meal the entire contents of a jar of food; therefore, the partially emptied jar may be stored in a refrigerator. Even under the best conditions there is a possibility of conversion of nitrate to nitrite. The tolerance level to nitrite is much lower than is the tolerance level to nitrate. There is no ready solution to this problem other than discarding partially-consumed jars of baby food.

CONCLUSIONS

Nitrogen fertilizer applied to vegetables tended to increase the nitrate content of the edible portion of these vegetables, except in the case of carrots. The magnitude and significance of this increase varied between species and in some species between years. The greatest effect of nitrogen fertilizer on nitrate content appeared to occur in the earlier-maturing species, especially radishes and mustard. A rate of 50 pounds of nitrogen per acre appeared to be a maximum rate to prevent marked nitrate accumulation in garden vegtables.

The omission of particular elements from a base treatment gave inconsistent effects on nitrate content. From the practical standpoint, producers should insure that the soil contains available phosphorus, since many of the vegetables grow early in the spring when soil temperatures restrict mineralization of soil phosphorus. The lack of phosphorus had an abvious effect upon rate of plant growth, even though the lack of sufficient phosphorus had little effect on nitrate levels within the plant.

The seasonal influence seemed to have more effect upon the nitrate content of vegetables than did maturity of the plant. These two effects were confounded with each other so that a concrete statement about either is not possible.

Insect infestation had profound influence upon nitrate contents, probably through the influence of the insects upon plant growth. The insect damage noted in the study was accompanied by a cool, wet spring; therefore, the actual cause-effect relationships could not be resolved.

The implications of the nitrate accumulations on human health can not be spelled out. There are no satisfactory guide lines to follow in making recommendations concerning consumption of fresh vegetables containing nitrates. This aspect awaits findings by the medical profession.

TABLE 28--A SUMMARY OF A SURVEY OF NITRATES IN PRODUCE AND BABY FOOD SOLD IN COLUMBIA, MO., GROCERY STORES BETWEEN JULY AND DECEMBER, 1964

Fresh Vegetable	Range in NO_3-N % in Dry Matter	State of Origin
Beans, snap	0.04-0.25	Calif.
	0.08	Colo.
	0.07-0.09	Fla.
	0.09-0.16	local
	0.08	Texas
Broccoli	0.01-0.09	Calif.
	0.08	Fla.
Brussel sprouts	0.01-0.06	Calif.
Cabbage	0.01-0.07	Calif.
	0.09	local
	0.01-0.03	Texas
Cabbage, red	0.01-0.19	Calif.
	0.22	Colo.
	0.02-0.16	Texas
Carrots	0 -0.13	Calif.
	0 -0.04	Colo.
	0.03	Texas
Cauliflower	0 -0.31	Calif.
Celery	0.11-1.12	Calif.
Corn	0.01	local

Fresh Vegetable	Range in NO_3-N % in Dry Matter	State of Origin
Cucumbers	0.02-0.13	Calif.
	0 -0.16	Fla.
	0.03-0.16	local
	0.01-0.15	Texas
Endive	0.14-0.36	Fla.
	0.06-0.67	Calif.
Lettuce, Bibb	0.44-0.60	Calif.
	0.65-1.05	Fla.
Lettuce, iceberg	0.14-1.05	Calif.
Lettuce, leaf	0.02-0.59	Calif.
	0.44	Colo.
	0.43-1.06	Fla.
	0.07-0.46	Mo.
Parsnips	0 -0.04	Calif.
Radishes icicle	0.41	Calif.
	0.65	Colo.
	1.33-1.54	Fla.
	1.01	local

TABLE 28 - CONTINUED

Fresh Vegetable	Range in NO_3-N % in Dry Matter	State of Origin	Baby Food Vegetable	Range in NO_3-N % in Dry Matter	State of Origin
Radishes, red	1.20	Calif.	Beans, green	0.04-0.29	unknown
	0.39	Colo.			
	0.80-1.12	Fla.	Beets	0.09-0.84	unknown
	0.79-1.05	Kansas			
	1.01	local	Carrots	0.06-0.30	unknown
	0.99-1.50	Mich.			
	1.01	Minn.	Corn	0.01	unknown
	0.95	Ohio			
			Peas	0 -0.02	unknown
Spinach	0.41-0.63	Fla.			
	0.07-0.66	Kansas	Potatoes, Sweet	0 -0.04	unknown
Squash, yellow	0.09-0.43	local	Spinach	0.11-0.82	unknown
Tomato	0.09	Ark.	Squash	0.01-0.13	unknown
	0.11	Calif.			
	0.04	Fla.			
	0 -0.04	local			
Turnips	0.03-0.76	Calif.			
	0.03-0.69	local			
	0.03	Texas			

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