T H E S I S

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TISSUE TESTING AS AN INDEX TO THE NUTRITIONAL STATUS OF CARNATIONS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY RALPH C. PETERSON

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Chapter I

INTRODUCTION

Soil testing is being used extensively as a means of regulating fertilizer programs for carnations, but this practice has serious limitations in the modern greenhouse where fertilization is accomplished by injecting dissolved fertilizer salts into the irrigation water. The nutrient reserve in the soil is small and not always a good indication of the nutrients available to plants. Soil nutrient levels maintained by dry applications of fertilizers are necessarily high and may reach excess proportions under a constant feeding program. Plant tissue testing, when correctly applied, will yield information which cannot be obtained in any other manner.

Holley (11) and Holley and Woodward (12) showed that lower than optimum nutrient levels produced adverse effects on both quality and yield of carnations. Also, no significant improvement in yield or quality of flowers was found with nutrient levels higher than this optimum (21). In fact, excess salts have been shown to adversely affect both quality and yield (27). The conclusion drawn from this later work was that the lowest possible level of soluble salts consistent with an optimum nutrient level should produce the highest flower yield and quality.

The problem

Can the analysis of selected carnation tissue be used to estimate accurately the nutritional status of the plant?

Problem analysis.--The problem has been divided into the following parts:

1. Can tissue testing be used to detect with accuracy the differences among carnation plants supplied with sub-optimal, optimal, and hyper-optimal nutrient levels?
2. Which carnation tissue can be used as indicators for the several nutrients?
3. How much accuracy can be sacrificed for speed and simplicity of analytical procedure?
4. What environmental factors are likely to affect the results of tissue tests?
5. Can tissue testing be adapted to commercial practice?

Delimitations.--This investigation was limited to five levels of nutrient solution on Pink Sim carnations from June 15, 1959, to July 1, 1960. The levels of fertilizer were established at 1/4 standard, 1/2 standard, 3/4 standard, 1 standard, and 1-1/2 standard rates. The investigation was carried out at the Colorado State University research greenhouse, Fort Collins, Colorado. The elements investigated were nitrogen, phosphorus, potassium and calcium.

Definition of terms.--Standard solution, as used in this experiment, refers to irrigation water which contains the following amounts of chemicals in each 175 gallons:

1.	Calcium nitrate	544 grams
2.	Sodium nitrate	24 grams
3.	Potassium chloride	160 grams
4.	Magnesium sulfate	48 grams
5.	Borax	5 grams
6.	Phosphoric acid (52%)	100 ml

These chemicals were dissolved in 5 gallons of water and injected into the irrigation water by a model PR Fert-O-Ject [1] proportioner.

The word nitrate is used to indicate the nitrate nitrogen which is reduced by sulfuric acid and indicated by diphenylamine.

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[1] Available from Fred C. Gloeckner & Co., Inc., New York 10, New York.

Chapter II

REVIEW OF LITERATURE

The concept of tissue testing and the use of foliar analysis to determine the nutritional status of plants is not a new one. Since the time of Liebig (14) men have considered the use of chemical diagnostic methods to increase their knowledge about the nutritional requirements of plants. With vastly increased knowledge of both chemistry and plants in the last three decades, the subject has received much concentrated effort by many workers as is evidenced by the wealth of literature.

Goodall and Gregory (7) have compiled a detailed review of literature pertaining to the composition of plant materials as an index to the nutritional status of plants. Their analysis suggested the integrated use of soil tests, field experimentation, and plant tissue tests as a solution to plant nutritional problems.

Broyer (2) discussed the previous work on the subject in a progress report designed as an introduction to the field. He presented in chronological order, discussions of the various phases and their development in conjunction with other fields.

Iljin (13) presented a critical review of the literature from 1850 to 1954, which included topics on soil factors affecting

availability of nutrients, the mechanism of ion absorption by plants, and soil and plant tissue analysis.

Other reviews have been concerned with more specific areas of research on the subject, such as Mitchell's review (17) of spectrographic analysis of plants and soils in which was listed some 260 references.

Practical work in the use of tissue testing received a great impetus when Hoffer (10) devised a system of tissue testing which furnished the basis for many further works dealing with interpretations of field and plot results. This system made use of colorimetric determinations. The system was later revised and extended by Thornton et al (25), and is widely known as the Purdue system. They described their field and laboratory techniques and reagents used to diagnose mineral nutrient deficiencies in plant tissue.

The Purdue test for nitrates (25) was made by applying a sulfuric acid solution of diphenylamine directly to freshly cut plant tissue. The intensity of the blue color which formed was a semi-quantitative measure of the nitrates present in the tissue. The phosphorus determination involved the use of ammonium molybdate, hydrochloric acid, and stannous chloride. A blue color was formed in the presence of phosphorus, the intensity being a measure of the phosphorus. Potassium was determined by the sodium cobaltinitrate method in which a precipitate was formed when potassium was present.

Thornton et al (25) stated that their system produced only semi-quantitive results and served only to detect trends. The extremes in plant nutritional status were easily detected but the intermediate tests were harder to interpret. The integrated use of plant and soil tests was again suggested.

Atkinson et al (1) used the Purdue (Thornton's) method for three years on tomatoes, corn, and potatoes. They found that tissue tests were dependable in showing where nitrogen and potassium fertilizers had been applied but could not depend on the results to indicate the increased use of phosphorus fertilizer. They found that nitrates and potassium slowly decreased in the tissue as plants advanced in age; that phosphorus increased as nitrate decreased; and vice-versa.

Spurway (23) presented and described a practical quick-test method of diagnosing soil fertility by means of simple chemical tests performed on soil extract. He described the procedures and reagents and published a set of color charts for use in interpreting the results. This system of soil analysis differed only slightly from the system used by Thornton; however, no reference was made to tissue testing by Spurway. Dilute acetic acid was employed as the extracting solution.

Peech and English (20) stated that many of the methods of soil and plant analysis do not consider interfering ions, hence these methods may be less reliable than they indicate. They devised a method of testing with more specific reagents which mask the effects of interfering ions in soil tests. Peech and English,

using Morgan's (18) universal extracting solution, did not test the reliability of their system on plant material. Morgan's extracting solution differs from Spurway's in that it makes use of sodium acetate as well as acetic acid.

Peech and English (20) used a considerably larger amount of ammonium molybdate in their phosphorus test than did either Spurway or Thornton, and added formaldehyde as well as ethanol in their potassium determinations. The formaldehyde reduces the error caused by ammonia in the plant or soil extracts. They described a brucine test for the determination of nitrates in preference to the diphenylamine test, which was used by both Spurway and Thornton. Calcium was determined by means of a soap solution containing stearic acid, oleic acid, ethanol and ammonium carbonate.

Harrington (8) used Peech's method on spinach and beans. He found that when any one of the three major elements (N, P, and K) was decreased on an experimental plot, the amount of that ion was lowered in the plant and the other two were increased. He also found that differences in ion concentration in the plant were caused by differences in age, fertilizer imbalance, and types of fertilizers used. He showed that care must be exercised in selection of the portion of the plant to be analyzed, and concluded that mature leaves constituted a more reliable sample than meristematic tissue. He also stated that samples from different plants must be morphologically the same to constitute comparable samples.

Lynd et al (15) also showed that the portion of the plant to be used must be selected with care, and expressed the belief that tissue testing could be made practical if comparable plants were sampled. They believed that some method other than visual selection should be used.

Thomas (24) has adapted several total analysis procedures to a few specific horticultural crops. He emphasized that these procedures had meaning only in a comparative sense, that is, compared with one another, and should not be considered as methods of assessing plant needs.

Emmert (6) found that tests measuring the soluble fractions of nutrients in plants were more accurate than those using total measurements. The percentage of difference was found to be greater in the soluble fractions than in the total amount of the element present. He also observed and discussed the same nitrogen-phosphorus relationship described by Atkinson above.

Danielson (4) stressed the importance of concurrent use of soil and tissue tests as a measure for unsatisfactory fertilizer conditions. He also stated that any observations of insect damage or visible nutrient deficiency symptoms must be carefully considered if the correct interpretations are to be made from the tissue tests. He describes his system of testing which makes use of modifications of the tests devised by Emmert (5) and Morgan (18), concluding that the use of tissue tests and soil tests together was very useful in preventing fertilizer deficiencies.

Hill and Cannon (9) stated that application of fertilizer in amounts assumed to be adequate is no assurance that the needs of the plant are being met. The sensitivity of the plant to its environment is the principle that makes tissue testing of value. These workers believe that the critical levels of the various fertilizers should be determined for the different horticultural crops.

Burriel-Marti' and Ramirez-Munoz (3) have presented a comprehensive book dealing with the theory and use of flame photometers and flame spectrophotometers in which they discuss the extent and limitations, sensitivities, and applications of flame photometry in the analysis of agricultural materials such as soils and plants. They also present their recommendations for setting up a flame photometry laboratory and suggest methods of analyzing samples.

The flame spectrophotometer has proved to be useful in the determinations of potassium and many of the minor elements in agricultural materials such as plant and soil extracts, and fertilizers. Calcium cannot be determined on the Beckman model B spectrophotometer because interferences cause erroneous interpretations. Nitrogen and phosphorus cannot be determined in this manner.

Chapter III

METHODS AND MATERIALS

Many investigators (4, 6, 7, and 20) believe that tissue testing offers a means of obtaining valuable information concerning nutritional status of plants. This experiment was designed to study practical tissue test methods for carnations as well as to obtain basic information concerning the effects of fertilizer treatments on the composition of carnation tissue.

General procedure

A special bench was constructed to support 30 boxes, 36 inches above the floor level of the greenhouse. Each box was 30 by 8 inches and 6 inches deep. Sufficient drainage was provided by drilling 12 holes, 3/8 inch in diameter, in the bottom of all boxes which were to contain soil. Scoria boxes were drained through holes cut in one end of the boxes, as near the bottom as possible. This end was then placed slightly lower allowing excess water to drain to it. Each box constituted one plot, the plots being separated by a space of 6 inches on the bench.

The growing media were soil and volcanic scoria. Scoria is an inert cinder which is devoid of plant nutrients, and sufficiently porous for good aeration while retaining more nutrient solution

than does fine gravel. The soil used was a clay loam with high organic content, similar to that soil used for growing carnations in the Colorado State University research greenhouse.

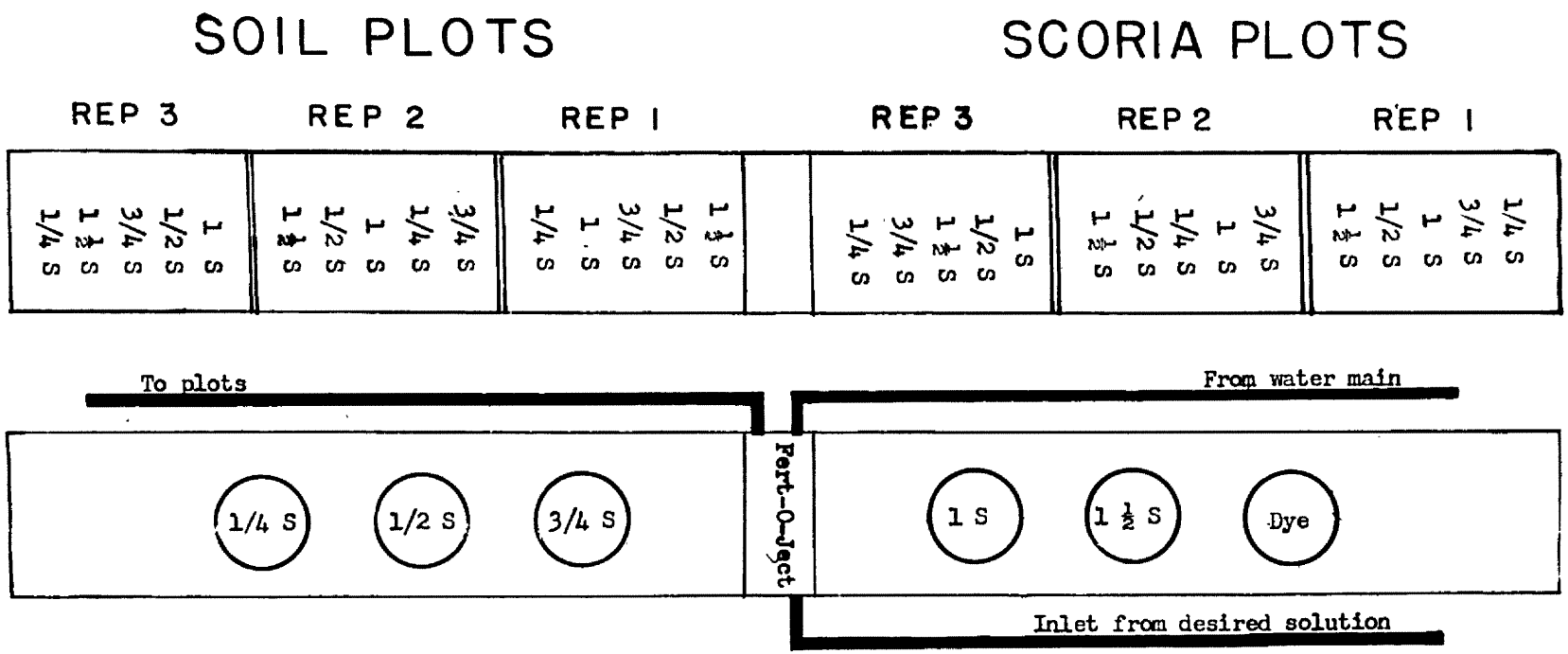
Scoria was selected for three replications in the experiment because it affords more precise nutritional control than does soil. Soil was used for three replications because it is the medium used in most commercial greenhouses.

Table 1.--Amounts of fertilizers used in five nutrient solution concentrations.

Chemical	Grams per 175 gallons				
	1/4 S	1/2 S	3/4 S	1 S	1-1/2 S
Calcium nitrate	136	272	408	544	816
Sodium nitrate	6	12	18	24	36
Potassium chloride	40	80	120	160	240
Magnesium sulfate	12	24	36	48	72
Borax	1.25	2.50	3.75	5.00	7.50
Phosphoric acid (52%)	25	50	75	100	150

Five levels of nutrient solution concentration were chosen for this investigation: 1/4 standard, 1/2 standard, 3/4 standard, 1 standard, and 1-1/2 standard (Table 1). Three replications of the five fertilizer levels on scoria occupied the east half of the bench and the same three replications with soil occupied the west half. All plots were in a random block design (Figure 1).

Figure 1.--A. Position of the five plots within the three replications on two media.
B. Method of applying nutrient solutions.



1/4 S - Standard nutrient solution at 1/4 strength.
1/2 S - Standard nutrient solution at 1/2 strength.
3/4 S - Standard nutrient solution at 3/4 strength.
1 S - Standard nutrient solution.
1-1/2 S - Standard nutrient solution at 1-1/2 strength.

All boxes of scoria and soil were steam heated to a minimum of 180° F. for one hour, then allowed to cool. Rooted carnation cuttings of variety Pink Sim were planted five inches apart along the longitudinal center of each plot on June 15, 1959. After the plants became established the terminal shoot of each was removed to promote branching.

A Lark tensiometer was placed in the 1 S plot of replication 2 in soil (Figure 1) and all plots were watered when a moisture tension of 300 cm water was registered in that plot. Observation indicated that this system of determining watering frequency was satisfactory. No mulch or covering of any kind was used on either soil or scoria in this experiment.

Application of water and nutrients

The scoria plots were originally designed to be subirrigated from bottles of nutrient solution which were below the bench. The solutions were forced up into the boxes by air pressure, then allowed to drain back. At the end of five months the method was discarded as being unsatisfactory. Particles of scoria plugged the solution lines resulting in uneven watering. Fresh solutions had to be made up for all plots after three waterings. The subirrigation equipment was removed and scoria plots were watered in the same manner as soil plots. Nutrient solutions in both cases were identical.

Irrigation water containing the desired amounts of nutrient solution was applied at each irrigation through a model PR Fert-O-Ject automatic fertilizer injector, a light weight, portable machine designed to operate at low flow rates. It is a positive displacement type injector with a fixed injection ratio of 1:35.

Nutrient solution storage tanks were 5 gallon, glass carboys placed beneath the bench. Five carboys contained the stock solutions of the different fertilizer solutions, while a sixth contained malachite green dye solution. The dye was used as a flush solution in the Fert-O-Ject between fertilizer treatments, since the same equipment was used for all treatments. White (26) used essentially the same method.

Selection of tissue for testing

Samples of several carnation tissues were selected: mature leaves, immature leaves, vegetative breaks, and reproductive breaks (Figure 2). The investigation was limited to tissue which could be removed from the plant without injuring it or reducing its ability to produce flowers. All tissue for any one sample was selected to be of uniform physiological age and condition.

The plants for this phase of the work were grown in scoria with 1 S nutrient solution. Tissue from the different locations were analyzed at least once a month for six months to determine which constituted the most reliable sample.

Figure 2.--Carnation stem showing:

1. Reproductive breaks
2. Vegetative breaks
3. Immature leaves
4. Mature leaves

Figure 2.--Carnation stem showing:

1. Reproductive breaks
2. Vegetative breaks
3. Immature leaves
4. Mature leaves

Preparation of plant samples

Plant samples were collected and taken to the laboratory where they were rinsed twice in distilled water to remove soil or other residue which could interfere with the test results. After rinsing, the samples were placed on a clean surface and cut into half-inch segments, following which a 10 gram randomly selected sub-sample was used for the tests.

The extraction processes chosen for study were: drying and grinding, freezing and expressing the sap, and extraction from fresh tissue with dilute acetic acid in a Waring blendor. Comparisons were made on the basis of accuracy, reproducibility, and ease and speed of handling.

Selection of analytical methods

A comparison of analytical methods was made on the basis of accuracy, speed, and compatibility with present methods of soil testing. Samples of carnation tissue were tested by the various methods. Test results and ease of handling were used as criteria in choice of methods. The methods studied most intensely were those described by Peech and English (20), Spurway (23), and Thornton (25). Modifications of these methods were also used to find the most satisfactory standard procedure. Potassium was determined by a model B Beckman flame spectrophotometer, an instrument which is extremely accurate for this purpose. The concentration of several minor elements may also be determined with this instrument.

The flame spectrophotometer was calibrated to give direct potassium readings in parts per million. This was accomplished by using a 10,000 megohm resistor, the red sensitive phototube, a sensitivity setting of 3, and light with a wave length of 768 m u. The exit slit was adjusted using a set of known standards such that the instrument could be read directly in parts per million. This slit setting was approximately 0.27 cm.

The standards were prepared by dissolving the proper amounts of potassium chloride for 100 ml standards in 20 ml 2% acetic acid and diluting to 100 ml. This was done to insure that the standards contained the same strength acetic acid as did the samples, since acetic acid tends to enhance the effects of potassium on the flame photometer.

Estimation of critical levels of nutrients in the tissue

The results of tissue tests made from the plants grown for this investigation were compared. Inferences were made from the data concerning the critical levels of nitrate, phosphorus, potassium and calcium. The critical level is defined as the level at which each element should be present in the tissue if the nutritional status of the plant is optimum.

Evaluation of the system

Plant and soil samples were collected from commercial carnation greenhouses and comparative analyses were made in an attempt to establish any correlation between the two types of tests.

Tissue was selected according to the standards set for collecting tissue and random soil samples were taken from the same benches.

Chapter IV

RESULTS

This investigation was designed to study the reliability of tissue testing as a means of determining the nutritional status of carnations. Previous experiments (12, 21) showed that quality and yield of carnations were significantly affected by the nutrient levels similar to those which were chosen for this work. Growth records were not a part of this experiment; however, the differences in growth caused by the various levels of fertilizer on plants grown in scoria are shown in Figure 3. The variation from one level to another in scoria was much more marked than it was in soil (Figure 4). The initial fertility of the soil was such that distinct levels of nutrients in the plants were not established. Growth differences were noticeable only on plants grown with the 1/4 S solution.

Use of composite samples

The use of composite samples reduced the number of tests necessary; therefore, most tests were made from samples of combined tissue from all three replications of each fertilizer level. The practice of using composite samples was also preferred because of the similar practice used to sample regular greenhouse benches, where the samples are composed of tissue gathered from many locations in the

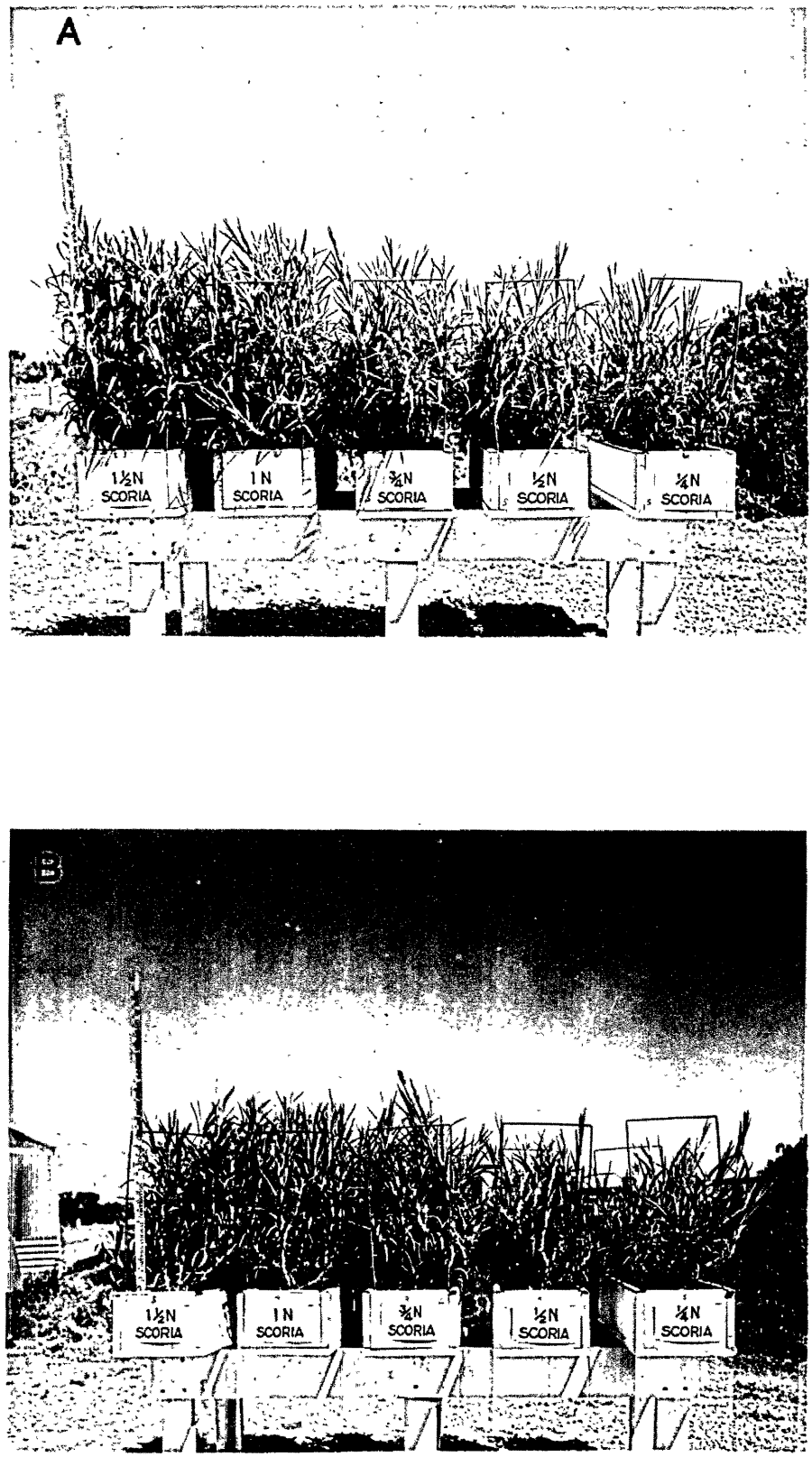


Figure 3.--Plots from replication1 in scoria, showing differences in amounts of growth due to fertilizer level.
A. South view
B. North view

* N is synonymous with S.

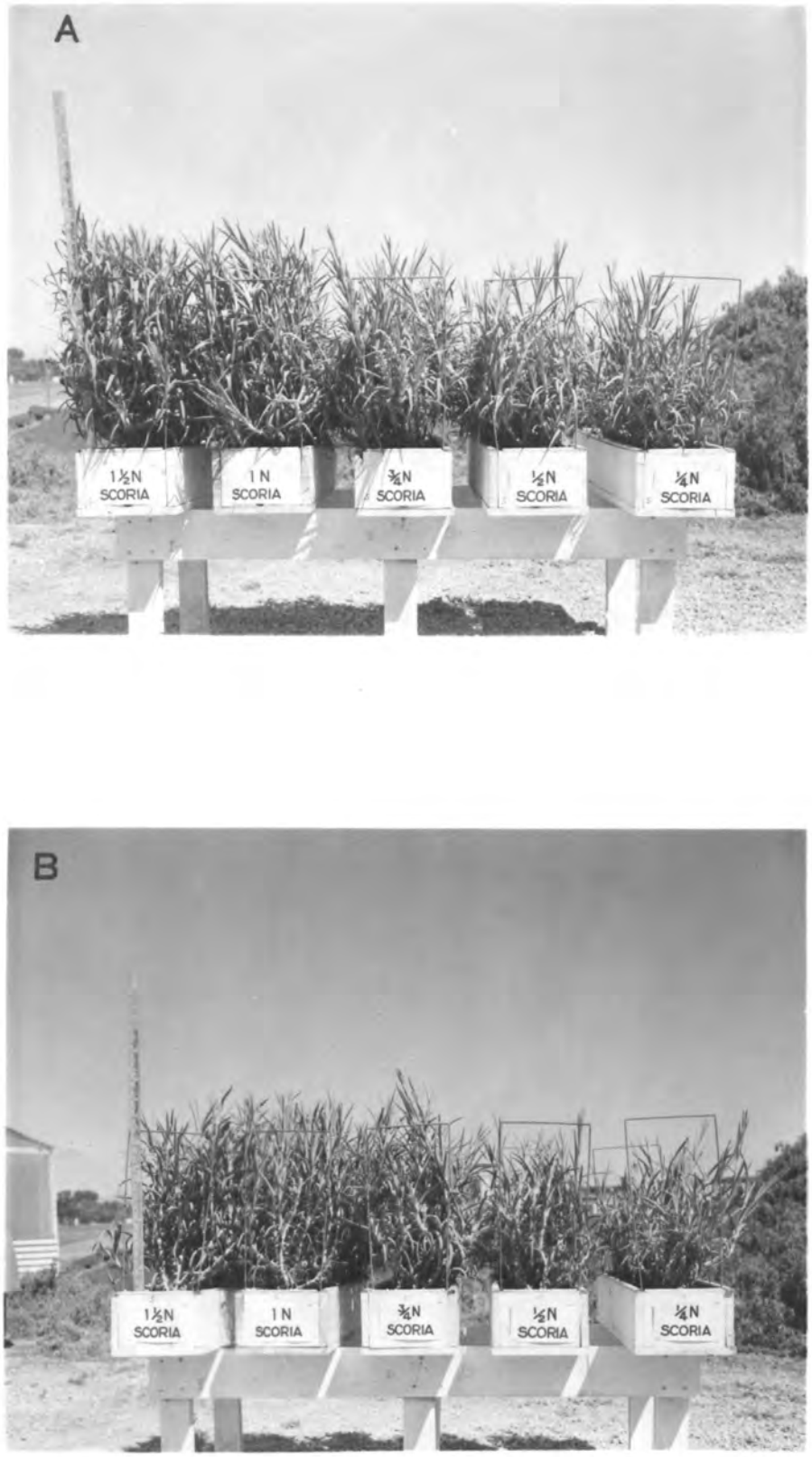


Figure 3.--Plots from replication1 in scoria, showing differences in amounts of growth due to fertilizer level.
A. South view
B. North view

* N is synonymous with S.

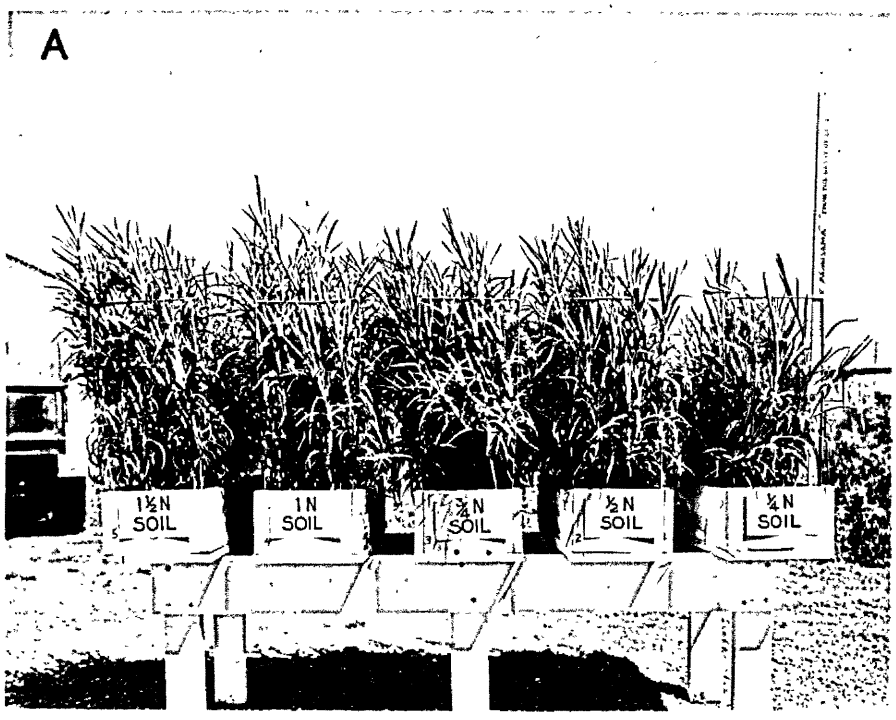


Figure 4.--Plots from replication 1 in soil, showing differences in amounts of growth due to fertilizer level.
A. South view
B. North view

* N is synonymous with S.

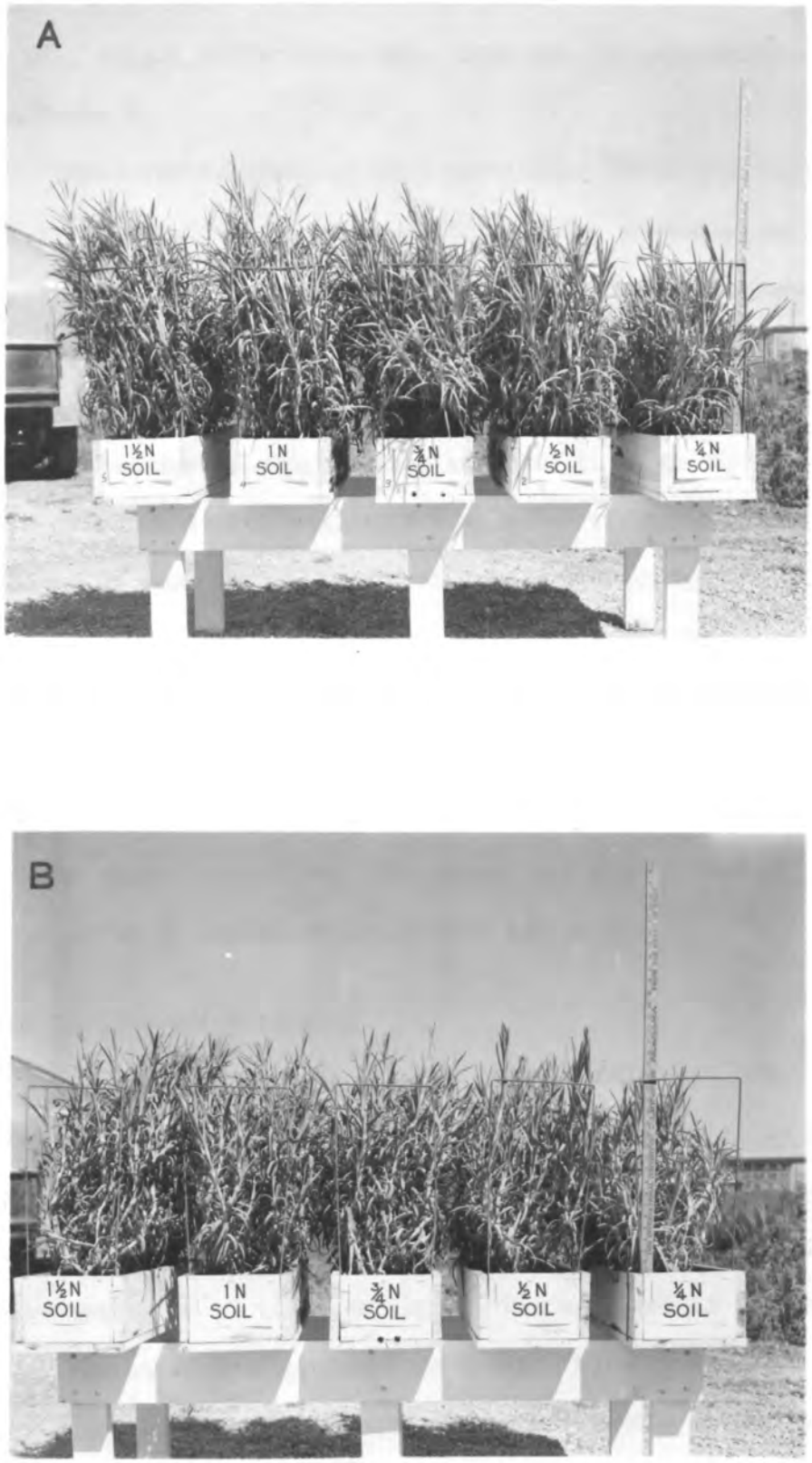


Figure 4.--Plots from replication 1 in soil, showing differences in amounts of growth due to fertilizer level.
A. South view
B. North view

* N is synonymous with S.

bench. Only slight differences were found due to replication as shown in Table 2.

Three replications of each fertilizer level were used in order to provide sufficient material for testing purposes, to indicate any differences due to position on the bench, and to safeguard against loss of material such as occurred with the 3/4 S plot of replication 2 in scoria. Comparisons of tests from these replications showed little variation due to replication, as shown in Table 2. It was therefore determined that composite samples of tissue from the three replications of each fertilizer level were satisfactory.

All data shown in Tables 3 to 14 are from composite samples of tissue from the three replications, with the exception of the 3/4 S level. This level in replication 2 received serious physical delay early in the experiment and sampling of this plot was avoided to eliminate error in results. The damage was due to lack of nutrient solution, caused by faulty subirrigation equipment.

Selection of tissue for testing

Tests of tissue made between January and July, 1960, showed that mature leaves from any of the three nodes directly below the cutting level of a carnation stem were the most reliable tissue for nitrate, phosphorus, and potassium determinations. The uppermost vegetative break, of 10 to 14 cm length, was selected as the best tissue for the calcium determinations (Figure 2).

Soluble nitrate was found in larger amounts in the less mature tissue as compared to mature leaves, but that in the younger

Table 2.--Amounts of nitrate, phosphorus, potassium, and calcium in extract from carnation plants grown in scoria at five levels of nutrient concentration.[a] Tests made June 27, 1960.

Replication	Fertilizer Level	ppm NO_3[b]	ppm P[c]	ppm K[c]	ppm Ca[b]
1	1/4 S	T[d]	5	32	100
	1/2 S	2	4	40	125
	3/4 S	5	4	47	125
	1 S	15	2½	55	150
	1-1/2 S	25	2½	76	150
2	1/4 S	T	5	35	125
	1/2 S	2	4	41	125
	3/4 S	missing	missing	missing	missing
	1 S	10	2½	51	150
	1-1/2 S	25	2½	81	150
3	1/4 S	T	4	33	100
	1/2 S	2	4	41	125
	3/4 S	5	2½	48	150
	1 S	15	4	54	150
	1-1/2 S	20	2½	78	150

Analysis of Variance

Source	d.f.	M.S.	M.S.	M.S.	M.S.
Replication	2	1.66	0.065	1.50	74.50
Fertilizer level	4	271.00**	2.225**	874.25**	948.71*
Error	7	4.28	0.060	3.25	72.50

[a] Mature leaves used for nitrate, phosphorus, and potassium tests; vegetative breaks for calcium tests.

[b] Ten grams of tissue in 200 ml 2% acetic acid.

[c] Ten grams of tissue in 200 ml 2% acetic acid; further diluted 1:5 with distilled water.

[d] Trace too small to measure accurately; calculated as 0.5 in analysis.

* Significant at 5 per cent level.
** Significant at 1 per cent level.

tissue varied with time of day and light conditions, making interpretations difficult. This source of error was found to be less marked in mature leaves. Amounts of nitrate present in mature leaves did not show differences due to time of year (Table 3).

Table 3.--Amounts of soluble nitrate found in carnation tissue from plants grown in scoria with 1 S nutrient solution.

Date	ppm nitrate in plant extract [a]			
	Mature leaves	Immature leaves	Vegetative breaks	Reproductive breaks
January 11	15	20	25	20
February 9	15	10	15	20
March 14	15	20	20	15
April 11	15	15	25	25
May 9	10	25	15	20

Since differences of 1 or 2 ppm nitrate at levels higher than 10 ppm are difficult to detect, a series of standards was made from known solutions of 0, 2, 5, 10, 15, 20, and 25 ppm nitrate and test results were compared and read to the nearest 5 ppm at levels higher than 10 ppm.

Mature leaves were found to give the most reproducible tissue test results and be more indicative of the nitrate level in the nutrient solution of the plants. Comparative tests from four tissues and five levels of nutrient solution in scoria are shown in Table 4. Tests at various times during the season showed similar results. The data for mature leaves are given in Table 5.

[a] Ten grams of tissue in 200 ml 2% acetic acid.

Table 4.--The nitrate content in carnation tissue from plants grown in scoria with five nutrient levels. Data collected April 11, 1960.

Nutrient level	ppm nitrate in plant extract /a			
	Mature leaves	Immature leaves	Vegetative breaks	Reproductive breaks
1/4 S	T /b	5	5	5
1/2 S	2	10	15	10
3/4 S	5	20	15	20
1 S	15	15	20	20
1-1/2 S	20	20	25	25

Phosphorus and potassium, like nitrate, were found in greater amounts in young tissue as compared to mature leaves. Also, tests made on immature tissue were less accurate as indicators of the nutritional status of carnation plants. Potassium and phosphorus levels in immature leaves were found to be less sensitive than nitrate to light conditions.

An inverse relationship was found between the amount of phosphorus extracted and the strength of the nutrient solution, and seemed to indicate that the phosphorus was not a limiting factor for plants in this experiment. Phosphorus levels are shown in Tables 6, 7, and 8.

Table 5.--Seasonal variation in nitrate found in mature leaves of carnations grown in scoria with five nutrient levels.

Nutrient level	ppm nitrate in plant tissue /a				
	March 14	April 11	May 9	June 23	July 1
1/4 S	T/b	T	2	T	T
1/2 S	2	2	2	2	2
3/4 S	5	5	5	10	5
1 S	15	15	10	15	15
1-1/2 S	20	20	20	25	25

/a Ten grams of extract in 200 ml 2% acetic acid.
/b Too small to measure accurately.

Table 6.--Soluble phosphorus extracted from various carnation tissues on different dates. Plants grown in scoria with 1 S nutrient solution.

Date	ppm phosphorus in extracts [a]			
	Mature leaves	Immature leaves	Vegetative breaks	Reproductive breaks
January 11	3	5	5	4
February 9	2½	3	4	5
March 14	2½	5	3	2½
April 11	3	4	5	3
May 9	2½	5	4	4

Table 7.--Soluble phosphorus extracted from various carnation tissues grown at five nutrient levels in scoria. Data collected April 11, 1960.

Nutrient level	ppm phosphorus in extract [a]			
	Mature leaves	Immature leaves	Vegetative breaks	Reproductive breaks
1/4 S	5	5	4	5
1/2 S	4	5	5	4
3/4 S	4	4	5	4
1 S	3	5	4	4
1-1/2 S	3	3	4	3

Table 8.--Seasonal variation in soluble phosphorus extracted from mature leaves of carnations grown with five levels of nutrient solution in scoria.

Nutrient level	ppm phosphorus in carnation extract [a]					
	Feb. 9	Mar. 14	April 11	May 9	June 23	July 1
1/4 S	5	5	5	5	5	5
1/2 S	4	4	4	4	4	4
3/4 S	4	4	4	4	4	3
1 S	2½	2½	3	3	2½	3
1-1/2 S	3	2	3	2½	2½	2½

[a] Ten grams tissue in 200 ml 2% acetic acid; further diluted 1:5 with distilled water.

Potassium extracted from carnation tissue was found to increase as the strength of the nutrient solutions increased; however, the lowest amounts of potassium found in the control plants was considerably higher than the lowest amounts found in commercially grown plants (Table 16). This would suggest that potassium, while not present in amounts equal to plant needs in the less concentrated solutions, was not the primary limiting factor. The primary limiting factor was probably nitrate. Reduced amount of growth of these controlled plants due to low nitrogen supply, probably resulted in the higher potassium content. Test results of the control plants are shown in Tables 9, 10, and 11.

Table 9.-- Seasonal variations in potassium extracted from carnation plants grown in scoria with 1 S nutrient solution. Potassium determined with a flame spectrophotometer.

Date	ppm potassium in carnation extract[a]			
	Mature leaves	Immature leaves	Vegetative breaks	Reproductive breaks
April 11	51	63	60	65
April 25	55	62	57	59
May 9	54	68	71	62
May 26	56	67	65	68
June 23	53	63	68	70

[a] Ten grams of tissue in 200 ml 2% acetic acid; further diluted 1:5 with distilled water.

Table 10.--Potassium extracted from carnation plants grown in scoria with five nutrient levels. Data collected May 26, 1960.

Nutrient level	ppm in plant extract [a]			
	Mature leaves	Immature leaves	Vegetative breaks	Reproductive breaks
1/4 S	38	52	57	56
1/2 S	42	55	60	60
3/4 S	46	58	63	59
1 S	56	67	65	68
1-1/2 S	76	76	78	74

Table 11.--Seasonal variations in potassium extracted from mature leaves of carnations grown with five nutrient levels in scoria.

Nutrient level	ppm potassium in plant extract [a]				
	April 11	April 25	May 9	May 26	June 23
1/4 S	30	36	33	38	32
1/2 S	42	41	43	42	39
3/4 S	49	47	45	46	48
1 S	51	55	54	56	53
1-1/2 S	75	78	83	77	80

Extractable calcium was not found to be greatly different due to nutrient level in either mature or immature tissue (Tables 12, 13, and 14). Less calcium was obtained from mature leaves than from immature tissue. As nutrient solution concentration increased, a more definite increase of soluble calcium was found in vegetative breaks than in other tissue tested. Thus tissue from vegetative breaks was selected as that giving the best indication of the calcium status of the plant. This was expected since calcium deficiency symptoms occur on immature tissue (16, 22).

[a] Ten grams of tissue in 200 ml 2% acetic acid; further diluted 1:5 with distilled water.

Table 12.--Seasonal variation in calcium extracted from four carnation tissues from plants grown in scoria with 1 S nutrient solution.

Date	ppm calcium in plant extract[a]			
	Mature leaves	Immature leaves	Vegetative breaks	Reproductive breaks
January 14	100	125	150	150
February 9	125	150	125	125
March 14	125	125	150	125
April 11	100	125	125	100
May 9	125	125	150	125

Table 13.--Calcium extracted from four carnation tissues from plants grown in scoria with five nutrient solutions. Data collected June 3, 1960.

Nutrient level	ppm calcium in plant extract[a]			
	Mature leaves	Immature leaves	Vegetative breaks	Reproductive breaks
1/4 S	100	100	125	100
1/2 S	100	125	125	100
3/4 S	125	100	150	125
1 S	125	125	150	100
1-1/2 S	125	125	150	125

Table 14.--Seasonal changes in calcium extracted from vegetative breaks of carnation plants grown in scoria with five nutrient levels.

Nutrient level	ppm calcium in plant extract[a]					
	Jan. 11	Feb. 9	March 14	April 11	May 9	June 23
1/4 S	100	100	125	125	100	125
1/2 S	125	125	125	125	125	125
3/4 S	125	150	150	150	125	150
1 S	150	125	150	125	150	150
1-1/2 S	150	150	150	150	175	150

[a] Ten grams of tissue in 200 ml 2% acetic acid.

Preparation of plant samples

Selected plant samples were subjected to three methods of extraction: drying and grinding, freezing and expressing the sap, and extraction with dilute acetic acid in a Waring blendor. Samples which were dried and ground had the advantages that they could be stored for an indefinite period of time and the results were slightly less variable than samples prepared in any other way; however, the amount of time and space required for this procedure made it impractical when a large number of samples were to be analyzed. For this reason the procedure was abandoned.

For the second method samples were frozen and the sap was pressed at a pressure of 1000 psi. This method is rapid but the results were variable and are not considered sufficiently accurate for this type of testing program. The results of sub-samples from the same sample were sufficiently different from each other that the technique was discarded. Differences may have been due to uneven pressures on the tissue within the pressing chamber or to differences in amounts of water in the tissue. Possible errors in the large dilutions necessary may have been a contributing cause.

Extraction with dilute acetic acid in a Waring blendor offered a method which was fast and produced an extract with little variability. Several variations of the procedure were investigated. These included adjusting the concentration of the acetic acid, time of extraction, and amount of activated carbon used as a decolorizing agent. The best results were obtained by using 10 grams of prepared

carnation leaves, and covering with 200 ml of 2 per cent acetic acid solution, adding .25 grams (1/4 level teaspoon) of Darco G-60 activated carbon and extracting for three minutes, then filtering through Whatman No. 1 filter paper.

When this method was used the extract was used full strength for the nitrate and calcium tests, and was diluted 1 to 5 with distilled water for the phosphorus and potassium tests.

Selection of analytical methods

The Spurway (23) soil test methods for nitrate and phosphorus were adopted because they were as fast and accurate as any other method investigated (20, 25) and are widely accepted in the United States. The Spurway methods of testing for potassium and calcium were not found to be satisfactory for tissue tests. Potassium was determined on a flame spectrophotometer, which is extremely accurate for this purpose. This method proved to be much more satisfactory than chemical methods.

The use of the flame spectrophotometer for calcium determination was not possible because of the interfering ions present in the plant extracts. The Peech and English (20) method proved to be the most reliable method although a considerable number of samples were tested by the versenate titration method (19). The titration method requires more time and equipment than the turbidimetric method (Peech and English), and was not found to be more accurate.

Estimation of critical levels of nutrients in the tissue

The estimation of critical levels of the various nutrients in plant tissue corresponds to the levels found in the tissue from plants grown at the optimum nutrient level (1 S), in scoria. The plant performance was such that these nutrient levels were considered optimum in the tissue (Table 15).

Table 15.--Critical levels of nitrate, phosphorus, potassium, and calcium in carnation tissue. Levels are expressed as parts per million in the plant extracts.

Nutrient	Low	Optimum	Excessive
Nitrate[a]	less than 10	10 - 20	Greater than 20
Phosphorus[b]	" " 2	2½- 4	" " 4
Potassium[b]	" " 50	50 - 70	" " 70
Calcium[a]	" " 125	125-175	unknown

Evaluation of the system

An evaluation of the system was made by obtaining 20 simultaneous tissue and soil samples from commercial greenhouses in the Denver, Colorado, area. Results of the tissue tests and corresponding soil tests appear in Table 16.

[a] Ten grams of tissue in 200 ml 2% acetic acid.

[b] Ten grams of tissue in 200 ml 2% acetic acid, further diluted 1:5 with distilled water.

Table 16.--Results of soil and tissue tests from commercial greenhouses in the Denver, Colorado, area. Samples were collected June 21, 1960, and analyzed June 22, 1960.

Sample Number	Tissue tests				Soil tests[a]			
	NO_3	P	K	Ca	NO_3	P	K	Ca
1	15	2½	59	150	5	2½	15	150
2	5	1½	51	125	25	½	20	150
3	2	5	40	150	20	1	10	200
4	20	5	44	125	15	5	10	200
5	25	½	50	100	25	½	15	200
6	25	1	38	125	15	½	5	125
7	10	½	33	150	5	½	5	125
8	15	5	41	150	15	5	10	150
9	20	½	58	150	15	1½	15	200
10	15	5	18	125	20	5	5	200
11	25	2	24	150	15	1	5	150
12	10	2½	50	150	5	2½	15	150
13	5	1½	52	150	10	2	20	200
14	15	2	50	125	20	1½	20	100
15	10	2	45	125	10	2½	15	125
16	5	1½	50	150	5	3	20	150
17	2	5	34	125	2	3	10	200
18	20	5	44	125	15	5	10	200
19	10	½	30	150	5	½	5	125
20	15	2	35	150	20	2	10	125

[a] Estimated optimum nutrient levels (Spurway) in soils for carnations being fertilized by constant injection of nutrients into the irrigation water: NO_3 = 10-20, P = 2½-4, K = 10-20, Ca = 150-200.

Chapter V

DISCUSSION

The combined efforts of agricultural chemists, soil scientists, and plant physiologists have resulted in many techniques for diagnosing the nutritional status of plants. The theories of nutritional diagnosis have been explored and set forth in such a manner that the way is now clear for other workers to investigate specific crops more thoroughly and accurately than ever before. A method of accurately estimating the true nutritional status of carnation plants at any given time can be of value to research workers in all areas of carnation study by providing better information on this evasive variable. The value of this information to the commercial grower is evident.

The need of being familiar with carnations before tissue tests are attempted should be stressed. Selection of tissue which is morphologically and physiologically identical with tissue tested at other times is of prime importance, as discussed by Lynd et al (15). During this investigation, tissue which showed evidence of insect or disease damage was avoided. Mature leaves were carefully selected from the plants. Only leaves from the cutting zone to about six inches lower on the stem were used (Figure 2). Where vegetative breaks are indicated, the uppermost vegetative break was selected when it was 10 to 14 cm in length.

Tissue tests which were made between January and July, 1960, indicated mature leaves were the most reliable tissue for nitrate, phosphorus, and potassium tests. The tests made from mature leaves showed considerable differences between fertilizer levels, but little difference within these treatments. The amounts of nitrate, phosphorus, and potassium in other tissue were more variable within treatments and less variable between treatments.

That mature leaves would be most indicative of nitrogen, phosphorus, and potassium levels was expected since these nutrients are mobile within the plant. When these elements become limited in the nutrient solution, they are translocated from mature tissues to help satisfy the needs of the younger tissues. A deficiency of any one of these nutrients should show first in the mature tissues.

Calcium is one of the elements which is immobilized by carnation plants. After calcium ions have been utilized by the carnation plant they are usually not available for translocation and reutilization. Calcium deficiency symptoms first appear on young leaves, in vegetative breaks and in the flowers (16, 22). It follows that changes in the calcium level of the growing medium should be first reflected in the immature tissue.

The method of extracting the soluble fraction of the nutrient elements from plant tissue must depend on the degree of accuracy desired and amount of time which can be spent on each sample. Test results are no more accurate than the extraction method, but the necessity for speed and accuracy must not be over-looked if

the system is to be practical for large numbers of samples. The dilute acid method on fresh tissue was chosen because it seemed to provide a compromise which met both criteria. Although dried samples seemed to give better results, too much time was required to make this method practical for commercial usage. Expressed sap was unreliable for testing because of the variation in results. Possibly the variability was caused by differences in the turgidity of leaves tested. The less turgid a leaf, the more concentrated the sap is likely to be. A more likely cause may be attributed to the lack of available solvent to flush out the nutrients. When dilute acetic acid was used, an excess of water was available to wash the soluble nutrients from the tissue, but when the tissue was pressed, only the plant sap was available for this purpose and a relatively large amount of the soluble elements may have remained in the tissue. Perhaps bathing the crushed tissue in distilled water or some other solvent would have removed these elements, but this reduces the main advantage of this system--speed and ease of handling.

Analytical methods were chosen by the same criteria as extracting methods, but with consideration for the soil test methods commonly used by commercial carnation growers. The chemical methods described by Spurway for testing soil appeared to be sufficiently accurate and rapid for determining nitrates and phosphorus in carnation tissue, but did not prove to be acceptable for calcium and potassium. Hence more reliable methods were utilized.

Soil used in this investigation contained so much reserve nutrients that it proved a poor medium for controlled nutrition. Nitrate was the only nutrient found deficient in any tissue from plants grown in soil. The tissue from plants which received 1/4 S solution showed an average of 5 ppm nitrate, and those which received 1/2 S solution showed an average of 10 ppm nitrate in the extract. The strength of the nutrient solution did not affect amounts of phosphorus, potassium, and calcium in the tissues grown in this soil. Soil test results were of the same general pattern, but nitrate differences were less distinguishable.

From the results of this investigation, nitrate was found to be the primary limiting factor, or the factor in relative minimum for plants grown in scoria. The amount of nitrate in the tissue varied significantly with the amount of nitrate in the irrigation water. Thus each treatment could be readily identified by results of the tissue tests. Soluble nitrate increased sharply as optimum level was approached. Both excesses and deficiencies were easily detected.

The amount of growth made by plants with solutions lower than 1 S decreased rapidly as strength of solution decreased. The reduced amount of growth accounts in part for the higher phosphorus content of tissue grown at the lower nutrient concentrations. The amounts of phosphorus present in the nutrient solutions was apparently sufficient to support the growth which was produced. Also many workers believe that the roles of nitrogen and phosphorus in plants are interrelated in a number of ways and that the availability of one may affect the availability of the other.

Potassium appeared less important than nitrate as a limiting factor, since extremely low levels of potassium did not appear in the plants grown at the lower levels. This may also be due to the decreased amount of growth made by these plants. Commercially grown carnations, known by soil tests and visable symptoms to be low in potassium, exhibited much lower amounts in tissue tests (Table 16). Excesses of potassium were evident in samples grown with 1-1/2 S nutrient solution.

Calcium was not found deficient or excess in any of the tissue tested in this experiment. The lack of low calcium readings in commercial plants was expected since carnation plants are normally grown at relatively high calcium levels. The lack of low readings in the control plants is probably due to the restricted amount of growth made, as in the case of potassium and phosphorus.

The results of tests made on commercially grown carnation tissue indicate the desirability of combined use of tissue and soil tests. Accurate interpretations of tissue test results are more nearly possible if the amounts of available nutrients in the soil are known. When phosphorus is high in the tissue, the excess is caused by high amounts of phosphorus in the soil, or low amounts of nitrate in the soil, or a combination of the two conditions. Accuracy of interpretation of any combination of tissue test results would be enhanced by accompanying soil tests.

Chapter VI

SUMMARY

Carnations were grown at five different nutrient solution concentrations. A nutrient solution giving optimum growth of carnations was diluted or concentrated to the following levels: 1/4, 1/2, 3/4, 1, and 1-1/2 standard strength.

Mature leaves, immature leaves, vegetative breaks, and reproductive breaks were collected from the plants grown at the optimum level in scoria and were tested for nitrate, phosphorus, potassium, and calcium content to determine which tissue was the best indicator of the plant's nutritional status. Mature leaves were selected for nitrate, phosphorus, and potassium tests; vegetative breaks for calcium. Drying and grinding, freezing and expressing the sap, and extraction from fresh tissue with dilute acetic acid in a Waring blendor were compared as methods of removing the elements from the tissue for analysis. The dilute acetic acid and Waring blendor method was selected for speed, accuracy, and adaptability to commercial practice. Comparison was made between the Spurway, Peech and English, and Thornton analytical methods. The Spurway tests were selected for nitrate and phosphorus tests; the Peech and English method for calcium. Chemical methods were not found to be reliable

for potassium tests so these determinations were made on a Beckman model B flame spectrophotometer.

Following the establishment of standard procedures, the nitrate, phosphorus, potassium, and calcium content of tissue from plants grown at the five fertilizer levels in scoria was investigated, and an attempt was made to establish optimum ranges for the soluble fractions of the four nutrients in carnation tissue.

Tissue testing can be used to indicate accurately the differences among carnation plants receiving sub-optimal, optimal, and hyper-optimal nutrient levels for nitrate, phosphorus, and potassium. No calcium deficiencies or excesses were detected by either soil or tissue tests.

This study suggests the use of both soil tests and tissue tests, if the maximum information about carnation nutritional status is to be obtained.

Different light intensities and time of day affected nitrate content of immature carnation leaves, but did not significantly affect nitrate content of mature leaves. Date of sampling or day length between January and May had no apparent effect on nitrate content of mature carnation leaves.

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ABSTRACT OF THESIS

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TISSUE TESTING AS AN INDEX TO THE NUTRITIONAL STATUS OF CARNATIONS

Submitted by

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In partial fulfillment of the requirements

for the Degree of Master of Science

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ABSTRACT

Soil testing is being used extensively as a means of regulating fertilizer programs for carnations, but this practice has serious limitations in the modern greenhouse where fertilization is accomplished by injecting dissolved fertilizer salts into the irrigation water. The nutrient reserve in the soil is small and not always a good indication of the nutrients available to plants. Soil nutrient levels maintained by dry applications of fertilizers are necessarily high and may reach excess proportions under a constant feeding program. Plant tissue testing, when correctly applied, will yield information which cannot be obtained in any other manner.

Comparison was made between the Spurway, Peech and English, and Thornton analytical methods. The Spurway tests were selected for nitrate and phosphorus determinations; the Peech and English method for calcium. Chemical methods were not found to be reliable for potassium determinations so these were made on a Beckman model B flame spectrophotometer.

Extraction with dilute acetic acid in a Waring blendor offered a method which was fast and produced an extract with little variability. Several variations of the procedure were investigated. These included adjusting the concentration of the acid, time of extraction, and amount of activated carbon used as a decolorizing agent. The best results were obtained by using 10 grams of prepared carnation leaves, and covering with 200 ml of 2 per cent acetic acid

solution, adding .25 grams (1/4 level teaspoon) of Darco G-60 activated carbon and extracting for three minutes, then filtering through Whatman No. 1 filter paper.

When this method was used, the extract was used full strength for the nitrate and calcium determinations, and was diluted 1 to 5 with distilled water for the potassium and phosphorus determinations.

Following the establishment of standard procedures, the nitrate, phosphorus, potassium, and calcium content of tissue from plants grown at five fertilizer levels in scoria were investigated, and an attempt was made to establish optimum ranges for the soluble fractions of the four nutrients in carnation tissue. The optimum levels in the plant extract are estimated to be:

Nitrate[1]	10 to 20 ppm,
Phosphorus[2]	2½ to 4 ppm
Potassium[2]	50 to 70 ppm
Calcium[1]	125 to 175 ppm.

Tissue testing can be used to indicate accurately the differences among carnation plants receiving sub-optimal, optimal, and hyper-optimal nutrient levels for nitrate, phosphorus, and potassium. No calcium deficiencies or excesses were detected.

An evaluation of the system was made by obtaining 20 simultaneous tissue and soil samples from commercial greenhouses in the Denver, Colorado, area. Tissue test results were compared to

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[1] Ten grams of tissue in 200 ml 2 per cent acetic acid.

[2] Ten grams of tissue in 200 ml 2 per cent acetic acid, further diluted 1:5 with distilled water.

soil tests in an attempt to establish any correlation which might exist. Although a correlation was evident, a combined use of soil tests and tissue tests was indicated.

The tissue test results from the commercial samples showed a wide range of variation above and below the estimated optimums, as follows:

Nitrate	2 to 25 ppm
Phosphorus	½ to 5 ppm
Potassium	18 to 59 ppm
Calcium	125 to 150 ppm.

Accurate interpretations of tissue tests are more nearly possible if amounts of available nutrients in the soil are known. When phosphorus is high in the tissue, the excess is caused by high amounts of phosphorus in the soil, or low amounts of nitrate in the soil, or a combination of the two conditions. Accuracy in prescribing corrective measures depends on this knowledge. Correct interpretation of any combination of tissue test results is enhanced by accompanying soil tests.

Different light intensities and time of day affected nitrate content of immature carnation leaves, but did not significantly affect nitrate content of mature leaves. Date of sampling or day length between January and May had no apparent effect on nitrate content of mature carnation leaves.