A Microchemical Study of Leaf Nitrogen in Lebanon During 1957 With Two Apple Varieties Grafted on Three Understocks

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Leaf Nitrogen in Apple Trees

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Abstract

A one-year study was conducted to determine microchemically the total leaf nitrogen during the 1957 growth season with Golden Delicious and Starking Delicious apple varieties grafted on three understocks, Malling VII, Malling IX and Malus communis. Five replicate of each of the six combinations were used from which representative samples were obtained. The leaf samples were collected at 8 different times during the growing season at 3-week intervals starting May 14 and terminating October 8, 1957. The trunk circumferences were measured at the beginning and at the end of the study to determine the increment. Both chemical and physical data obtained are presented in 25 tables and 6 graphs. The analysis of variance for total nitrogen in the leaves shows that significant differences occured in the following comparisions: (1) between dates of sampling, (2) stock-scion combinations with Starking Delicious/Malling IX being consistently the lowest, (3) varietal effect in that Golden Delicious was significantly higher irrespective of the stock used. The understock effects, in general, were not significantly different, and (4) the Golden Delicious is significantly higher than the Starking Delicious. The analysis of variance for the increase in trunk circumference shows that: (1) the Starking Delicious made a significantly higher increase than Golden Delicious, and (2) the understock effects were the same statistically in that they did not have an influence on the scion varieties except for Malling VII where Golden Delicious was the scion variety. The estimate of the coefficient of correlation between the total nitrogen in the leaves and the increase in the trunk circumference is negative and is significantly different from zero.

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Introduction

The apple, which is the second most important tree fruit produced in Lebanon, is grown extensively in the irrigated, mountainous areas and in the Bekaa plain. Within recent years the development of the apple industry has proceeded rapidly and with little previous investigation as to the basic requirements for long-time successful production.

Golden Delicious and Starking Delicious are the two leading commercial apple varieties in Lebanon at present and both appear to be well adapted. Little or no study of understocks has been made to date in Lebanon. One of the most common rootstocks used is Malus communis. Malling VII and Malling IX have been suggested as having desirable characteristics and qualities suitable to local conditions and a number of orchardists have used them to a limited extent as understocks. The scion-rootstock interaction in horticulture (24) is now widely recognized and one of the tools used to study this influence is the course of change of total nitrogen in apple leaves during the growing season of a given period.

In contrast to the long established soil analysis methods, chemical analysis of the leaves from different parts of the plant has been accepted as a diagnostic tool in the nutritional studies of fruit trees. This has been extensively studied by several investigators located in different geographic areas (2,18,27,30,43).

"Foliar or leaf analysis," as defined by Thomas and Mack (50), "is the chemical composition of a leaf with respect to the dominant nutritive 'element' (entities) at the moment of sampling, taken from a predetermined and suitable position". This means that the chemical foliar analysis is a sequence of the chemical conditions of the selected leaf sample as determined, at the different periods, during the investigation.

Plants derive most of their nutrition from the available elements in the soil, and it is mainly in the leaf "where the raw materials from the soil and air are synthesized into assimilable vital compounds from where they are redistributed throughout the plant to build the new-wood and the fruit tissues" (26). Based upon this, the leaf is considered the best part of the plant to be analyzed due to its vital importance in the nutritional processes of the plant (26).

Since nitrogen is one of the major limiting elements for growth and productiveness of the apple trees in Lebanon, a knowledge of its seasonal uptake and distribution is essential for a sound fertilizer program.

Although a large number of analyses have been performed at particular dates (37), which vary with the different investigators, yet few reported the seasonal changes of nitrogen in the leaves, and much interest has been focused toward the changes taking place during the autumnal yellowing of the leaves (12). Hence it would be desirable to take several samples during the season in order to determine how much the plant is supplied with nitrogen during its season of growth. If the leaves are sampled early in the season, they show a relatively high percentage of total nitrogen, while if sampled at the end of the season, the percentage of nitrogen will be relatively low due to the accumulation of carbohydrates, a slower rate of nitrogen intake from the soil and the withdrawal of nitrogen from the leaves prior to abscission (5,12,26,28,37).

The differences in the results obtained by the various workers are mostly due to variation in the climatic conditions and the solar radiation (32).

After the establishment of scientific interpretation of the results of foliar analysis and the setting of limits designated as "optimum values" to every element needed, the mineral composition of the leaf is coming to be accepted as a valid criterion for the nutritional status and fertilizer requirement of the plant. For it was shown that the composition of the leaf will determine how well the roots are able to uptake the nutrients as well as the nutrient needs of the trees, irrespective of the mineral nutrient supplies in the soil (19).

The present study was initiated at the beginning of the 1957 growing season. The objective was to investigate the course of change of total nitrogen in apple leaves during one growing season. An orchard was selected in which both Golden Delicious and Starking Delicious scions had been grafted on Malling VII, Malling IX and Malus communis stocks.

Review of Literature

De Saussure in 1804 was the first to analyse the ash content of plants and found that the composition varied with the age, the part sampled and the soil conditions (58). Weinhold in 1862 and 1864 formulated the idea that plant analysis is an index of the available nutrients supplied; this thought is based on the assumption that the locations, where the plants are growing naturally in abundance, will have the best soil fitted to supply the nutrients needed (19). Later, Hellriegel in 1867 and Wolff in 1868 conceived of soil fertility as a sort of book-keeping where the nutrients removed by the crops should be restored to the soil either directly or indirectly, as reported by Ulrich (58).

Hall, at Rothamsted in 1905, concluded that "though the straw (of barley) show very considerable fluctuations in its potash content, it is not always possible to interpret the results" (58).

Following these early investigations, the studies of plant tissues became more refined and scientific.

Nitrogen enters through the root system as a solution of the salts of nitric acid, such as $NaNO_3$ or KNO_3; sometimes it also enters in the form of ammonia. The rate of penetration of these compounds varies with the temperature and the moisture content of the soil, being greatest in the late spring and early autumn, but persisting throughout the growing season. This inorganic form of nitrogen is translocated, through the xylem and phloem of the trunk and the branches, to the leaves where the various bio-chemical processes elaborate it into amino acids and various other nitrogenous products (17), of which the various forms in the tree are the following: soluble nitrogen, protein nitrogen, basic nitrogen, amide nitrogen, alpha-amino nitrogen, polypeptide nitrogen, acid-insoluble humin nitrogen, combined ammonium nitrogen, nitrate nitrogen (52) and others of less universal occurence such as the alkaloids, ptomaines, amines, cyanogenetic glucosides and indican (18). Of these, the proteins are by far the largest quantities (30). The amino-acids combine to form the proteins of the protoplasm; the purines and the pyrimidines combine to give the nucleic acids, the nucleins and the nucleoproteins which are compounds characteristic of the cell nucleus (18).

The continued elaboration of the amino-acids proceeds and reaches a maximum level when the light, the soil and the moisture conditions are at optimum. The newly formed nitrogenous compounds are constantly being removed from the leaves and used for tissue build-up and repair, shoot growth, new leaves, increment to branches, trunk and roots, new roots and especially for fruit and seed development (18).

In the fig tree, in the spring when a tree is at its peak of activity, the translocation of large amounts of nitrate and the subsequent reduction takes place in the leaves. So the seasonal curve of the nitrate-nitrogen shows a drop in the spring with usually a further decrease throughout the season (33); this was also noticed in the apple leaves (18) and the greatest decrease occured in September in the apple, cherry, pear, and plum leaves, prior to their abscission (30).

In the apple there is a seasonal translocation from the woody parts, to the leaves and back, of nitrogen, minerals and certain organic substances (12). The movement from the leaves to the twigs, limbs, trunk and even to the root system takes place late in summer (after the tree enters the rest period) and continues till the leaf abscisses (18,28), at which time the nitrogen on the dry weight basis will drop to one percent or even less (28).

In the spring, this translocation is reversed and the woody parts supply nitrogen, minerals and certain organic compounds before the roots start supplying them to the young developing buds, for their growth and tissue formation (18,28).

Practically all the nitrogen in a tree is in the organic form and most of it is in the insoluble proteinacious materials. Stuart (5) showed that only when excess nitrogen fertilizers are supplied that NO_3^- and NH_4^+ are found in apple leaves or other tissues above the fibrous roots. Thomas (48) has shown that nitrogen is changed to amino-acids in the rootlets of the apple tree.

Batjer *et al.* (3) reported the absorption of nitrogen by the apple roots during the dormant stage, as nitrate and ammonium, and these were changed to the organic form in the roots, when the temperature was as low as 32 or 33^{o} F. In the orchard, this does not always happen because of a low supply of oxygen to the roots caused by the filling of the soil spaces with water, thus inactivating the roots. But the leaching of the soluble forms of nitrogen, by the percolating water, will make them come in contact with the roots and thus be passively absorbed, and made available for use when the new season of growth starts.

Several workers (5,30) have determined the nitrogen content in the apple leaves and their results show that it varied directly with the supply of nitrogen fertilizers. Others have demonstrated that with the advance of age of the leaves, there is an actual migration of nitrogen, accumulating in the twigs, branches and even trunk and roots of the tree (5,12,37).

In autumn, the changes in the nitrogen content of the apple leaves, senescent or yellowing, are the result of two yet overlapping causes; one is the downward movement or return of nitrogen or carbohydrates from the leaves to the woody parts, which is used for the first growth the following spring, and the second is the slight upward or intake movement of this element into the roots from the soil (28,30). If, for any reason the trees are defoliated prior to their natural drop, the transfer of nitrogen and carbohydrates will not be completed, resulting in a reduction in the stored nutrients in the wood, which explains why a weak start will take place in the following season of growth (30).

Murneek and Logan (30) referred to the work of Rippel who considered the coefficient of reabsorption for various plants to be 69 percent and believed that most of the nitrogen is in the form of protein. Schultze and Schutz as reported by Murneek and Logan (30) believed that there is a delay in the formation of nitrogen compounds rather than an evacuation and their loss was due to respiration.

When leaf analysis is used as a criterion for the nutritional condition of a fruit tree, the various factors should be considered besides those related directly to the availability of the element to the plant. These are (a) the type of rootstock, (b) the size of the crop, (c) the extent of pruning and (d) the injury to the tree, bothbiological and mechanical. These should be clearly studied before an attempt to interpret the results can be attained (17).

Changes in the nutrients in the leaf should not be interpreted as changes in the uptake of that particular element from the soil (8) and when the percentage of an element is calculated, it is on the dry weight basis due to the diurnal changes in moisture content (7).

When the more productive trees were compared with the less productive ones, their leaf nitrogen was higher, and it decreased with the advance of age (27).

Tip-, mid- and base leaves responded similarly to a decrease in the plant nitrogen throughout the investigation conducted by Emmert (13). The findings of Thomas (48) show that the leaves sampled from non-fruiting shoots were not always higher in nitrogen, phosphorus and potassium, throughout the growth period, than were the leaves of the same physiologic age from fruit bearing shoots. He concluded that the difference between these values is small and frequently not exceeding the analytical error; while Emmert (14) got results showing that "leaves from bearing trees were higher in nitrogen in all but three orchards". The size of the crop influences the nutritional condition of the tree and it was found that the leaves from a non-bearing season have generally lower nitrogen, calcium and magnesium, a higher phosphorus and potassium concentration,(17), and almost double the size of the leaves from bearing spurs (62).

Just after flower fertilization (about May 1st), the total quantities of nitrogen in the developing fruit rises very fast at the beginning. In fact, the developing fruit is the chief organ that draws the available nitrogen of the bearing spur for its development throughout the growing season. "However it is more likely that soon after its inception, the fruit dominates the nitrogenous metabolism of the bearing apple spur system, if not also a part of the branch to which the spur is attached" (29). Therefore, in the analysis and interpretation of the nitrogen distribution in the bearing spurs, the fruit crop must be taken into account (29); the flowers and the fruits constituting a drain on the vital compounds of the plant, such as the amino-acids and the carbohydrates, for the reproductive functions (17,19,21,40,62).

With the frequent samplings during the growing season, it is easier to follow the course of change taking place in the concentration and nutrition of the element or elements studied in the leaves, than with fewer samples (48,58). "Four to five samplings, at intervals of two to three weeks are most satisfactory", as expressed by Thomas (48).

The part of the plant sampled for the analysis should reflect its general condition with respect to that particular element or elements under investigation (58). This can be normally expected because leaves of the same physiologic or metabolic age and morphologically homogeneous, taken from plants of the same species growing under identical environmental conditions, undergo the same physiological processes and give identical foliar diagnoses, when sampled at the same time of the day (10,48,50); and furthermore the sample should be as uniform as possible, in that the leaf petiole and stems should be separated from the blades (58), and when sampling, one leaf from a terminal twig selected at random, is enough at any sampling time (2). The leaves should be free of disease or insect damage (22), and taken immediatly to the laboratory for processing (16) which includes cleaning, drying and grinding of the leaf material, for which several, more or less similar, techniques have been described by various workers (4,6,10,12,14,17,18,19,28,30,33,43,44,46,47,51,54,61).

Taylor (50) feels that the valid interpretation of leaf analysis data, as a diagnostic tool, depends not only on the use of proper sampling and analysis techniques but also on the appropriate methods of preparing the samples prior to analysis.

For the size of each sample two points should be considered; the first is that enough leaves should be removed to be a representative sample of the tree under consideration, and the second is that the number of the sampled leaves should not constitute a severe pruning operation which will upset the physiological and chemical processes of the plant (20).

The nitrogen content of the apple leaves begins to decline as soon as active growth stops and the most rapid drop occurs at the yellowing stage. The data, obtained by Thomas (48), show an increase in the one- or two-year old wood from September to November.

Murneek and Logan (30) referred to the work of Lincoln, and Lincoln and Benett who suggested that the amount of nitrogen reabsorption into the wood depends on the nitrogen content of the tree in that the higher the nitrogen in the tree the less the absorption prior to their drop, and vice versa. Murneek and Logan (30) believe that weather, such as sunlight and temperature, is an important factor governing the rate and the time of reabsorption of the organic compounds from the leaves into the woody parts; a cool atmosphere will hasten the process while an early frost will kill the leaves.

During the past few years, a new concept has been formulated which maintains that there is a certain "optimum value" for every element in the leaf tissue in order to have optimum growth in terms of yield and other factors; these values hold over a wide range of climatic and soil conditions. This concept seems to be more valuable than the determination of "critical values" below which deficiency symptoms are expected to show on the fruit, leaf or tree. Once these "optimum values" are determined in an area, they can be used as standards with which to compare the results of trees grown in that particular region, in case these trees are weak or showing deficiency symptoms, and then altering the composition of the fertilizers in order to attain these "optimum values" (45). This will involve two problems; the first is the accurate scientific determination of these "optimum values" for each element for the particalar crop; and the second is the determination of what, when and how much fertilizer is needed to bring the leaf concentration to these "optimum values", having in mind the differential rates of fixing of the elements by the soil (45,49). These "optimum values" can be determined by the use of extensive factorially-designated fertilizer experiments for several years and several samplings all through the growing season (45). Another alternative would be a scientific survey of leaf concentrations in good productive plants, for several sampling dates, all through the growing season, and for several years. The reason for having several samples during the growing season is to be able to compare the values obtained with the "optimum values" found at that particular date, i. e., not to compare values obtained from September samples with those for May or June (45).

Although these "optimum values" are not fixed - they are approximates only - they have proved to be satisfactory in practice when used as standards in diagnosing deficiencies on trees when the symptoms were not defined (45), because "the lack of any symptoms of malnutrition in no way indicates the existence of optimum nutritional status in the plant" (42).

Different investigators (6,18,28,29,39,43,44,53) have reported, more or less, the same values for nitrogen and most of these values are expressed as percent of the dry material of the leaf, no consideration being given to either the weight of the dry matter at each sampling or the number of leaves taken from each plant at each sampling date. The value obtained should not differ much from one tree to the other under the same conditions of management and treatment, otherwise there would be "no justification for considering mean samples" (50).

Shear et al. (42) consider that the analysis for one element by itself "may lead to erroneous conclusions", and the evidence accumulated from the work on tung trees, shows that the supply of elements other than nitrogen, may alter the composition of the leaf tissue, much more so than a change in the supply of nitrogen itself.

"One of the first general reviews of rootstock/scion relations was that of Hatton in 1930", as reported by Rogers and Beakbane (38).

There is a general tendency for a given rootstock to produce trees having the same general appearance and size when grown under similar conditions of climate, management and soil; this was found to hold true for Malling XII and Malling XVI on theone hand, and Malling VII and Malling IX on the other. The former group gives large trees, while the latter always produces dwarfish budlings (9,55,56,57).

Lincoln (23) "views the root or stock generally as a positive acting reproducible thing determining its own formal disposition rather than a pliant batch of tissues awaiting external influences to give it form. Thus, it has inherent tendencies for development and funtioning. In contrast with this view are some American observations showing that the scion influence is the decisive agent determining the formal characteristics of apple tree roots", while Roberts (35) says "not onlydoes a scion induce a root development which is typical for the variety and thus different from another variety, but it also causes the miscellaneous seedling roots to throw out root system which are remarkably uniform within the variety..... and in striking contrast to the marked influence which the scion variety has upon root formation in the case of seedling piece root grafts, there seems to be practically no such influence in the case of vegetatively propagated dwarfing stocks".

There is a great influence of the scion on the stock: a strong growing scion will cause corresponding growth in the rootstock, as compared to the latter if it were grafted to a slow growing scion. The exact reason is not known, but it is thought that the strong growing scion will give vitamin B_1 or an increase due to the larger area of photosynthetic processes as a result of a greater leaf area (10). Also a strong scion seems to cause the roots to be tolerant to unfavorable soil conditions that the same stock would not have if the scion were a weaker growing variety (9).

The recent work of Vyvyan (59), in 1955, has shown that although the growth of the "composite tree" is a compromise between the understock and the scion, the influence of the former on the relative growth is greater than that of the latter. "The dominance of rootstock influence was confirmed by McKenzie in 1956, not only for effect on growth, but also for effect on anatomical structure,"as reported by Rogers and Beakbane (38). This does not imply that the scion has no effect, but means that the understock modifies the scion behavior more than the scion modifies the rootstock behavior.

Rootstocks are known to exert profound effect upon several aspects of plant behavior. In general, they influence the scion variety in the following ways: early or late bearing, quick or delayed flowering, time of fruiting, size and quality of fruit crop, longevity, time of maturity, height and size of tree and resistance or susceptibility to insects or diseases (10,34,35,57). Also in mature trees, there is a differential ability of the stock to absorb and translocate nutrients, a differential ratio of leaves to whole tree, fruit yield to tree size, distribution of nutrient among tree parts and carbohydrate accumulation in certain parts of the tree and in the type and quantity of the plant regulating subtances present in the tissues of the shoot (38,60). Thus Malling XII induces the highest content of magnesium, boron and iron, and Malling IX the lowest; Malling V was intermediate in this respect in Cox's Orange Pippin leaves, when this variety was on the mentioned understocks (39). These different effects are mainly due to the supply of the mineral elements by the stock to the scion. Wallace et al. (60) found these same effects on citrus trees. Smith et al. (44) also found that the mineral composition of the orange leaves showed different trends on different rootstocks when planted under identical growing conditions. Haas, and Neff et al. also got similar results as reported by Thomas and White (47). Thus it can be seen that the stock affects various scion varieties unequally; one variety may prove successful on one stock while on another it is a failure (35).

The increase in size of the scion when budded or grafted on the stock is influenced by a number of factors such as (a) the environmental effect on the scion and on the rootstock, (b) the interaction between the stock and the scion, and (c) the influence of the environment on the new individual or "stion" as Webber designated such a combination (57).

Presently there is a great tendency to have small trees in the commercial orchards as compared to the big trees used in the past (57), the reason being the economic conditions which theoretically, at least, favor trees which can be planted closer and be more easily harvested, pruned, sprayed and thinned. In addition small trees come into bearing earlier and if the varieties are undesirable, the trees can be removed without as much loss.

Materials and Methods

The orchard, used in this study, is located in a recognized apple growing area at Khonsharah (Mount Lebanon) having an approximate altitude of 1000 meters, sloping northward. A few miles to the east is mount Sannin (2628 m.) and in the other directions are the adjacent mountainous areas having various elevations. In the winter season, the orchard suffers from strong winds, especially the southern one. The soil of the orchard is sandy clay loam; it was translocated from an adjacent hill in 1954, and some lime was added to raise its pH from (6.2 - 6.8) to (7.4 - 7.75). The surface drainage is excellent, but the subsoil drainage is not very efficient due to the under-ground seepage. No rock or excessive stones are present. Before setting the trees, the area had not been used for commercial orcharding, but was swampy and had naturally growing pine trees. The apple trees were set using the triangular system (3 m. apart), on terraces 10 m. wide and with about 120 cm. difference in height.

The training of the trees was according to the modified leader system. Pruning was done twice during the 1956 season, first in March and later in July as summer pruning. But in the 1957 season, only winter pruning was done. These were executed under the supervision of the owner of the orchard, and were somewhat severe in order to avoid possible damage from strong winds in the region.

From the beginning to the end of the study, the spray schedule was approximately the same, as follows: Delayed dormant: Orthocide (Captan 50) at the rate of 5 lb./100 liters of water and three days later, Folidol 10 gr.,and lead arsenate 200 gr. per 20 liters of water. At the pink stage Metasystox(20 gr.), Cupravite (200 gr.) and wettable sulfur (200 gr.) per

20 liters of water. At the petal fall: Folidol and wettable sulfur as before. From then on, the orchard received the same spray materials as used in the petal fall spray every 15 to 20 days, throughout the season, making a total of seven sprays.

The orchard was irrigated six times during the 1957 season at 15 to 18 day interval. The amount of water used was enough to wet the top 50 to 70 cm. of the soil using the basin system. The irrigation water contained pig urine.

During the 1957 winter season, every tree received one bag (about 15 kg.) of a mixture of cow, pig and goat manure. During the last week of September 1957, each tree received 6 tins (about 25 kg.) of pig manure.

Five replicates of the following six combinations of trees were used in this study:

Variety	Understock
Golden Delicious	Malling VII
Golden Delicious	Malling IX
Golden Delicious	*Malus communis*
Starking Delicious	Malling VII
Starking Delicious	Malling IX
Starking Delicious	*Malus communis*

The trees chosen for this study were located on two adjacent terraces in the middle of the orchard, growing in parallel rows. After discarding the weakest and the strongest, only the normal trees were used for the randomized selection. Each tree was then given a reference number and the trunk was marked, at 30 cm. from the soil level, with a paint to measure the increase in trunk circumference at this height during the 1957 season of growth (to the nearest millimeter).

Only one tree of the following combinations bore a few fruits:

Combination	Nº of fruits
Stark. Del./Malling VII	1
Stark. Del./Malling IX	2
Stark. Del./Malling IX	3
Stark. Del./*M. communis*	9

The leaf sampling was started on May 14 and was continued, at intervals of three weeks till October 8, resulting in eight samples taken from each tree during the season. Thomas (48) considers that four to five samplings at two to three weeks intervals would be most satisfactory.

At sampling time, rigorous care was taken to get representative random leaves of the same physiological age (31,48), from the central half of the shoot throughout the tree. These leaves "have proved to be the most satisfactory for a diagnostic approach to the nutrition status of fruit trees" (6). For greater uniformity, all the leaf samples were collected between 9 and 10:30 a.m. at any sampling date. The petioles were removed from the blades (58). Then the blades were placed in cheese cloth bags and were brought directly to the laboratory for processing and drying (10,20), because "leaching is more rapid from dead than from living leaves" (25).

Not more than one leaf was taken from any terminal at any one time (2,14,17); the leaves were free from disease, distortion, malformations, any sign of disorder (nutritional or otherwise) (17) or any visible adhering foreign material which may be a source of contamination such as soil or bird excreta.

The size of the sample was limited by two factors; first that sufficient leaves remain on the tree for subsequent sampling, and second that the amount of the removed leaves should be such as to avoid severe pruning, so that the chemical and physiological condition of the tree would not be altered (20). Eighteen to 20 leaf blades per tree were considered a suitable sample.

In the laboratory, the leaves were washed with 0.3 N HCl for about 10 seconds (for each sample), they were gently rubbed against each other and were rinsed several times with distilled water (25). Taylor (46) showed that the several cleaning procedures he used, did not leach any soluble material from the leaves and concluded "there was no significant difference in the concentration of any of the macro-nutrient elements".

After shaking off the excess water, the samples were placed in numbered cheese cloth bags and dried quickly in a forced-draft oven, at 70 ± 1^{o} C for 24 hours (4,17,31,48), so that the water was rapidly removed from the immediate vicinity of the samples as recommended by Piper (31), and to prevent,as much as possible, any loss by the respiratory processes (19,31). This operation was followed by grinding the dried leaf sample in a Wiley mill with a 60 mesh sieve (4) for greater ease in manipulation as well as to secure greater uniformity in composition (19,30,53). The samples were stored in screw-top sampling bottles, and before the analysis, they were dried in a vacuum oven at 70 ± 1^{o}C for 12 hours, and cooled in a dessicator before a sample for the nitrogen determination was taken (10). Each sample was treated separately and duplicate analyses were made. The results were calculated on the dry weight basis (16) due to the diurnal variation in the moisture content (7).

The method for determining the total nitrogen was the modified Kjeldahl-Gunning procedure to include the nitrates and the nitrites (1,54).

Reagents

A. Sulfuric acid (conc. 93 - 96%) free of nitrate and ammonium sulfate, containing 33.3 gr. of salicylic acid per liter.

B. Catalyst composed of:

a) Two parts of potassium sulfate

b) One part of copper sulfate.

C. Sodium thiosulfate crystallized (50 - 70 mgm.)

D. Sodium hydroxide (40 percent)

E. Boric acid (2 percent)

F. Hydrochloric acid - standardized

G. Indicator composed of :

a) 0.125 % methyl red solution

b) 0.083 % methylene blue solution

all dissolved in 95% ethyl alcohol.

Apparatus

A. Electric digestion rack

B. Micro-nitrogen distilling apparatus

C. Kjeldahl digestion flasks - 30 ml.

D. Micro-burette

Procedure

Transfer 20 - 30 mgm. of oven dried ($70 \pm 1^{o}C$ under vacuum) leaf material wrapped in a cigarette paper into a 30 ml. Kjeldahl digestion flask, add 2 ml. of the sulfuric-salicylic acid, swirl gently for some 30 minutes, stopper and allow to stand over night. Add an average crystal of sodium thiosulfate and place the flask on the raised digestion rack. Heat for five minutes and let cool. Add the copper sulfate-potassium sulfate from the edge of the spatula to the flask, heat for 30 minutes (until no more white fumes of SO_3 escape) rotating the flask every few minutes. Then lower the digestion rack to allow the content of the flasks to boil gently.

Continue this boiling until the solution clears (water white) and no carbonaceous particles stick on the sides. Allow to cool.

Add 15 to 20 ml. of distilled water, rotate the flask and allow to cool before starting the distillation.

In the receiving 125 ml. Erlenmeyer flask, put 10 ml. of the 2% boric acid, 15 ml. of distilled water and two drops of the indicator, so that the tip of the condenser is below the surface of the liquid.

Transfer quantitatively the digested material to the distillation flask of the apparatus, to which are added 10 ml. of the 40 percent sodium hydroxide followed directly by 10 ml. of distilled water, stopper tightly and resume heating in the steam generator chamber. Continue distillation for 5 minutes after the indicator changes its color (from light blue to green) in the receiving flask. Rinse the tip of the condenser with distilled water before titration. Titrate with standard hydrochloric acid using a micro-burette. The end point is the change from green to light blue.
Run a blank with every set of samples, and calculate the percentage total nitrogen as follows:

$$\frac{(D - B)(Na \times En) \times 100}{Ws}$$

where

D = ml. in determination

B = ml. in blank

Na = normality of the acid

En = equivalent weight of nitrogen

Ws = weight of sample in mgm.

When any duplicate results had a relative error of more than 6 percent, the analysis was repeated until it was equal to or less than 6 percent, i.e. in equational form:

$$\frac{X - Xm}{Xm} \times 100 \leqslant 6$$

where

X = percentage total nitrogen in sample

Xm = mean percentage total nitrogen in sample

The methods of statistical analysis used in this study were the analysis of variance and the coefficient of correlation.

The analysis of variance is based on the assumption that "the experimental errors to which the data are subject are independently and normally distributed with the same variance, the latter restriction being the most important" (36).

In the tables, the calculated F value is the quotient of the mean square of the combinations over the mean square of the error in the analysis of variance method of statistical analysis. The upper number following the letter F is the number of degrees of freedom of the combinations and the lower one is the number of degrees of freedom of the error of the experiment. If the value of the calculated F exceeds the respective 0.05 tabulated F, it is significantly different at 0.05 (*); if it exceeds the respective 0.01 tabulated F, the difference is highly significant at 0.01 (**).

The critical difference for the means is obtained by the formula:

$$c.d. = \frac{2S^2}{n} \times t$$

where

S^2 = mean square of the error

n = number of replicates in each combination

t = tabulated value in the "t" distribution table with the same degrees of freedom as the error at the respective 0.05 and 0.01 levels.

If the difference between any two means of different combinations is less than the critical difference, then the two means are in the same statistical group, at the respective levels; if it is higher, then they are in different statistical groups.

Results and Discussion

The chemical and physical data are presented in tabular and graphic forms for the various combinations and dates for the total nitrogen in the leaves and for the increase in the trunk circumference.

The figures used for total nitrogen are percent of the dry weight and those for the increase in the trunk circumference are in centimeters. The following abreviations are used to denote the various combinations:

Abreviation	Variety	Understock
G/7	Golden Delicious	Malling VII
G/9	Golden Delicious	Malling IX
G/MC	Golden Delicious	*Malus communis*
S/7	Starking Delicious	Malling VII
S/9	Starking Delicious	Malling IX
S/MC	Starking Delicious	*Malus communis*

These tables and figures show clearly and conveniently the seasonal trend in the nutritional concentration taking place in the leaves (30,58), and the increment in the trunk girth during the 1957 growth season.

As the leaf bud opens and the leaves start to unfold and begin to grow, there is generally a continuous decline in the percent of the total nitrogen; although in absolute quantity, it is actually increasing.

Table 1 shows the seasonal total nitrogen in the leaves collected at the various dates of sampling and for every replicate of the six combinations used in this study. The results obtained follow the same trend as found by Gardner *et al*. (18) who in connection with the distribution of nitrogen in the leaves say that " there are two periods of rapid decrease in the total nitrogen, one in May and the other in September....

Table 1. Seasonal total nitrogen in Golden Delicious and Starking Delicious apple leaves (percent dry weight) on Malling VII, Malling IX and Malus communis understocks sampled during the 1957 season

Combination	May 14	June 4	June 25	July16	Aug. 6	Aug. 27	Sept. 17	Oct. 8	Mean
G/7 1	3.53	3.20	3.45	3.41	3.20	2.71	2.98	2.72	3.15
2	3.55	3.44	3.48	3.04	3.20	2.82	2.84	2.49	3.11
3	2.92	3.38	3.55	3.19	2.91	2.46	3.00	2.68	3.01
4	3.08	3.36	3.21	2.69	2.92	2.89	2.91	2.68	2.97
5	3.30	3.44	3.47	3.14	3.03	2.69	2.96	2.87	3.10
G/9 1	3.52	3.17	3.33	3.20	2.89	2.50	2.92	2.80	3.04
2	4.06	3.45	3.38	2.95	2.94	2.64	2.99	2.75	3.14
3	3.37	3.22	3.50	3.24	3.23	2.63	3.08	2.89	3.14
4	2.89	3.05	3.38	2.94	3.04	2.67	2.87	2.67	2.94
5	3.98	3.34	3.46	3.13	3.11	3.03	3.09	2.96	3.26
G/MC1	3.33	3.57	3.29	3.09	2.98	2.87	3.09	2.70	3.12
2	3.38	3.21	3.56	3.29	3.21	3.16	3.13	3.08	3.25
3	3.08	3.49	3.72	3.45	3.22	2.98	3.29	2.79	3.25
4	3.49	3.43	3.66	3.21	3.20	3.11	3.22	2.64	3.24
5	2.75	3.06	3.41	3.16	3.11	3.02	3.26	2.67	3.06
S/7 1	3.79	3.29	3.06	3.16	2.98	2.72	3.04	2.44	3.06
2	3.57	3.10	3.45	3.21	3.02	2.69	2.84	2.66	3.07
3	3.49	3.09	3.12	3.07	3.03	2.48	2.59	2.47	2.92
4	3.52	2.93	3.18	2.90	2.95	2.90	2.80	2.95	3.02
5	3.38	2.92	2.96	2.99	2.56	2.59	2.67	2.84	2.86
S/9 1	3.21	3.02	3.15	2.92	2.52	2.41	2.52	2.47	2.78
2	3.53	2.70	2.94	2.93	2.53	2.40	2.66	2.37	2.76
3	3.14	2.78	2.84	2.79	2.38	2.10	2.25	2.28	2.57
4	3.23	3.16	3.06	3.09	2.87	2.86	2.63	2.63	2.94
5	3.61	2.70	2.98	2.99	2.54	2.38	2.60	2.42	2.78
S/MC1	3.12	3.43	3.34	3.21	3.01	2.78	2.90	2.86	3.08
2	2.98	3.06	3.23	3.20	2.91	2.55	2.88	2.54	2.92
3	3.30	3.17	3.14	3.17	2.88	2.62	2.65	2.61	2.94
4	3.49	3.23	3.24	3.14	2.61	2.67	2.80	2.77	2.99
5	3.68	3.11	3.13	3.05	2.73	2.52	2.47	2.52	2.90

Between these two periods of rapid decrease, the percent composition of the leaf is fairly constant". This was also found by Murneek and Logan (30), Mori and Sakamoto (27) and Boynton (5) on apples. In the grape petiole, the change is the same except that the "actual values are much lower than most deciduous fruits" (11). The combinations G/7 and G/MC were exceptions to the general rule in that the total nitrogen increased till June 25. Following this date, all the six combinations show a more or less rapid decrease till August 27, at which time there was a temporary accumulation of total nitrogen reaching a peak on September 17, after which the depletion was very rapid. Thus the return of nitrogen to the tree seems to start a long time before the end of the vegetative season. This was also found by other workers (18,28).

As seen in table 1, the first period of decrease, in the percent of total nitrogen, is when the leaf is growing very rapidly and the available nitrogen, in the tree, is found in a limited quantity and supply; the shoot and the roots are simultaneously growing rapidly and vigorously.

The second period, that is the period after September 17, shows another withdrawal of the total nitrogen from the leaves prior to their abscission. The investigators seem to agree on the fate of the removed elaborated nitrogen from the leaf, and some have shown that it passes to the twigs, branches and even to the roots (5,12,37), and is stored for the following spring growth (30). This explains why it is advisable to delay the pruning of dormant trees till the end of the winter season by which time the compounds removed from the leaves, prior to their abscission, will be translocated to the woody parts of the tree.

Figures 1 to 6 and table 1 show that the total nitrogen in the apple leaves between the two dates, June 25 and September 17, decreases for two main reasons: (1) the leaf does not receive an excess of elaborated nitrogenous compounds needed for tissue growth, storage and fruit formation (in case any is on the tree), and (2) the increase of the carbohydrate compounds in relation to the nitrogenous ones due to the photosynthetic processes in the leaf tissue. This period does not fully agree with the findings of Gardner et al. (18) who reported that the supply was fairly constant, as far as total nitrogen was concerned.

In table 1, the differences between the highest and the lowest values for the various means, in the different combinations, are almost the same and relatively small, although the combinations having the Malling IX understock have more dispersed mean values for their average seasonal total nitrogen.

Figures 1 to 6 show in a graphic form the course of change in the average total nitrogen in the leaves for every combination at the various dates of sampling. The seasonal curves portray more accurately the level of any particular element than the data obtained for samples taken at any one time during the growth season.

Any particular coordinate represents the mean of the different values obtained in the five replicates at that particular date of sampling, as reported in tables 1 and 18. So an ascent or descent in the curve, with time, is an indication of the supply to and demand by the plant, and the "relative level of the graph indicates whether the supply is high or low" according to Thomas and Mack (50). Therefore a downward gradient indicates a greater demand in relation to the supply of total nitrogen, while an upward one is an indication of accumulation and lack of utilization "because of inhibition within the plant" as explained by Thomas and Mack (50).

The several samplings show clearly and conveniently the seasonal

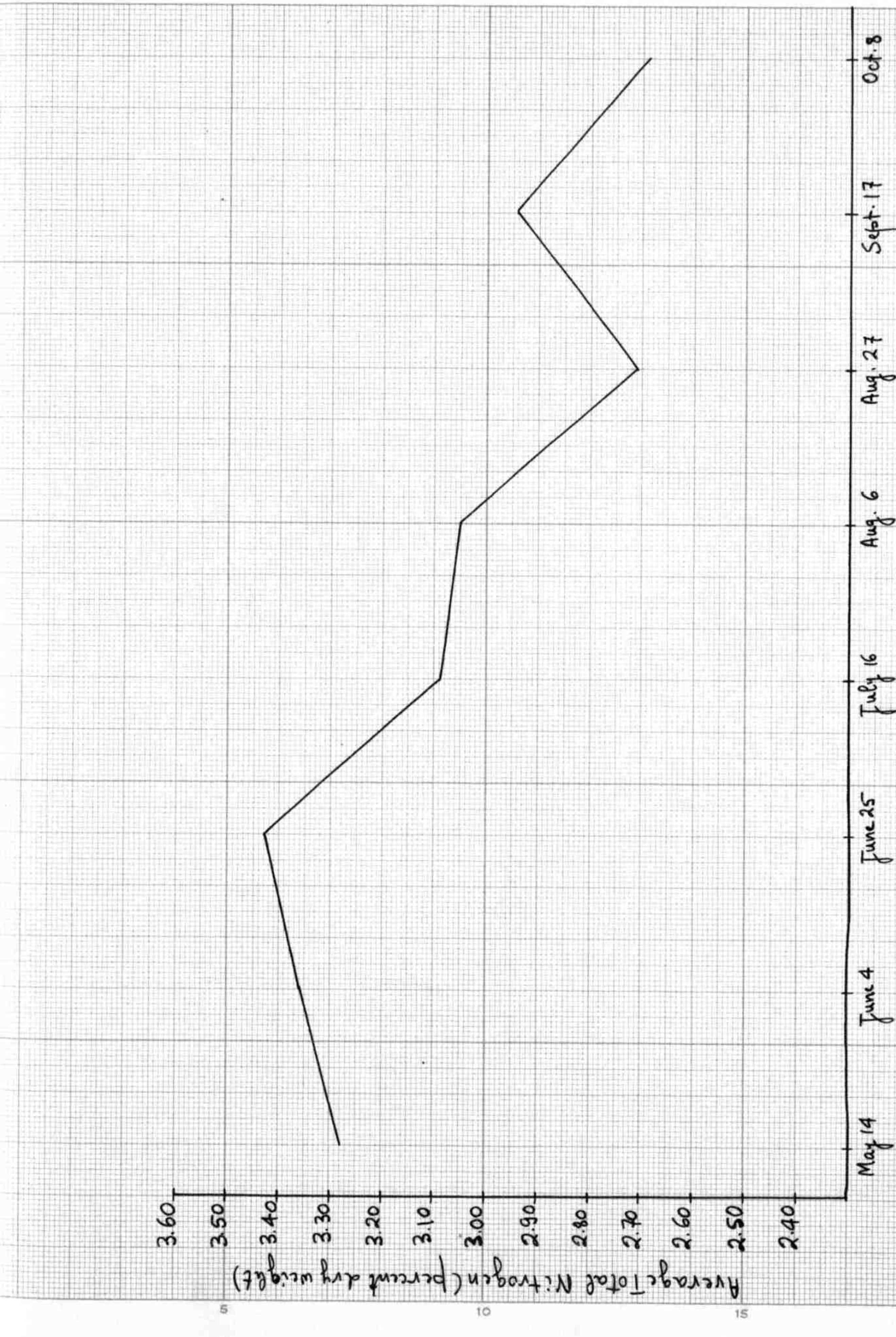

Fig. 1. Golden Delicious / Malling VII : Average Seasonal Total Nitrogen in Leaves

Fig. 2. Golden Delicious/Malling IX: Average Seasonal Total Nitrogen in Leaves

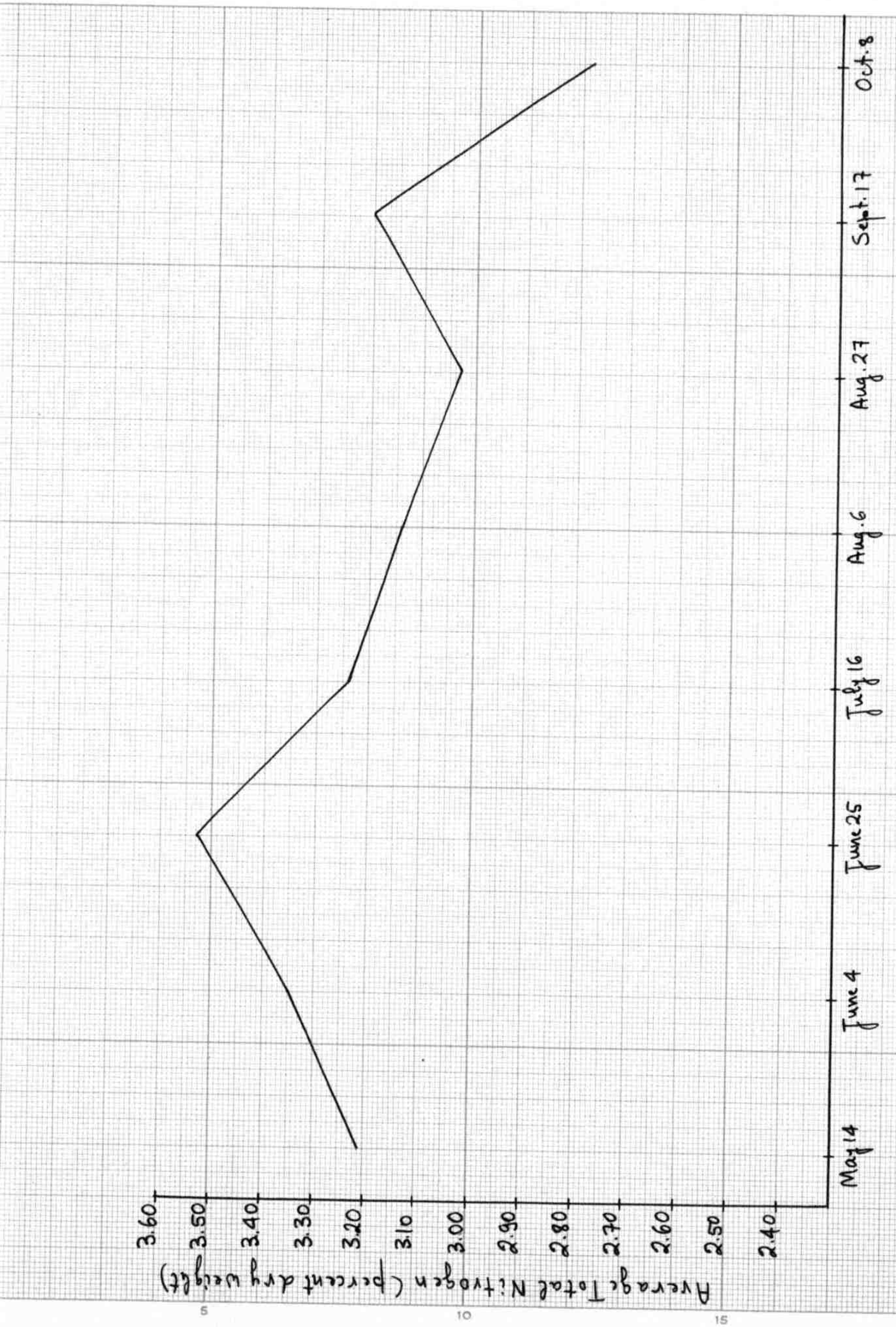

Fig. 3. Golden Delicious/M. communis: Average Seasonal Total Nitrogen in Leaves

Fig. 4. StarKing Delicious/Malling VII: Average Seasonal Total Nitrogen in Leaves

Fig. 5. StarKing Delicious/Malling IX: Average Seasonal Total Nitrogen in Leaves

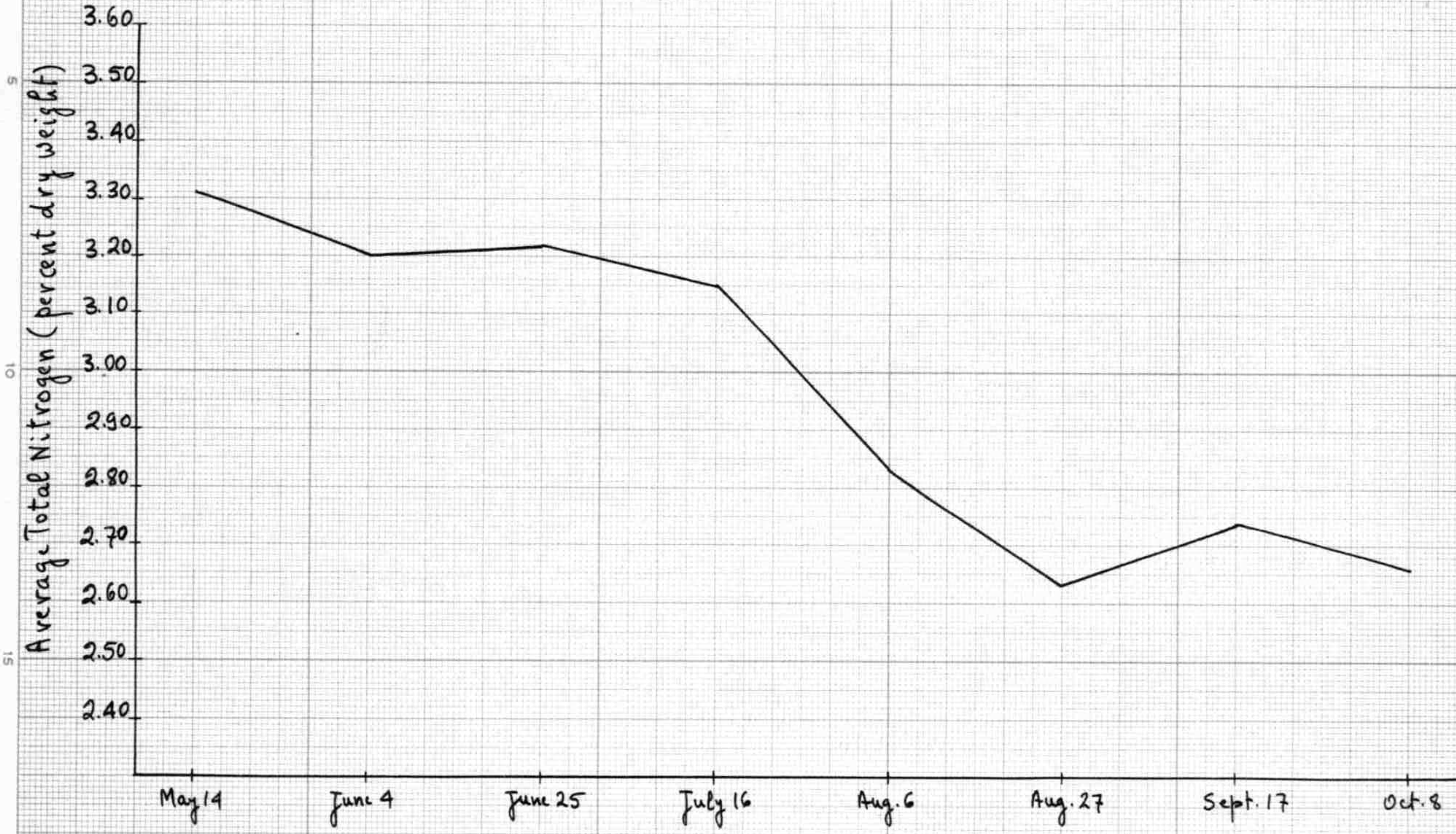

Fig. 6. StarKing Delicious/<u>M. communis</u>: Average Seasonal Total Nitrogen in Leaves

trend in the concentrations of the total nitrogen existing in the leaves during the season.

The Golden Delicious combinations show more abrupt changes in total nitrogen than those of the Starking Delicious.

"Leaf analysis showing unusually high or low nitrogen content should immediately direct attention towards the determination of the nutritional unbalance responsible for the situation", (42), and it is freely admitted that the results in one place do not indicate the conditions of other trees in other places.

By studying the results, in figures 1 to 6 and table 1, it is seen that all the percent total nitrogen values are higher than the reported ones in the literature (6,18,29,39,43,44,53). This could be explained by the following:

a. The large supply of nitrogen in the manure used and in the irrigation water; various investigators found that the nitrogen content in the apple leaves varies directly, within limits, with the quantity of nitrogen applied (3,5,16).

b. The climate, the soil and the solar radiation may be one of the more important variations influencing the composition of the leaves (32).

c. Nitrogen may be accumulating in the leaves due to a funtional unbalance existing between nitrogen and some other elements and the "lack of any symptoms of malnutrition, in no way indicates the existence of optimum nutritional status in the plant" (42).

d. The fact that many investigators reported their values at the mid or end of the season, at which time the total nitrogen, is at its minimum (24).

Table 2. Total nitrogen in apple leaves (percent dry weight) sampled May 14, 1957.

Replication	Combination					
	G/7	G/9	G/MC	S/7	S/9	S/MC
1	3.53	3.52	3.33	3.79	3.21	3.12
2	3.55	4.06	3.38	3.57	3.53	2.98
3	2.92	3.37	3.08	3.49	3.14	3.30
4	3.08	2.89	3.49	3.52	3.23	3.49
5	3.30	3.98	2.75	3.38	3.61	3.68
Mean	3.28	3.56	3.21	3.55	3.34	3.31

Calculated $F_{24}^{5} = 1.21$

Table 3. Total nitrogen in apple leaves (percent dry weight) sampled June 4, 1957

Replication	Combination					
	G/7	G/9	G/MC	S/7	S/9	S/MC
1	3.20	3.17	3.57	3.29	3.02	3.43
2	3.44	3.45	3.21	3.10	2.70	3.06
3	3.38	3.22	3.49	3.09	2.78	3.17
4	3.36	3.05	3.43	2.93	3.16	3.23
5	3.44	3.34	3.06	2.92	2.70	3.11
Mean	3.36	3.25	3.35	3.07	2.87	3.20

Calculated $F_{24}^{5} = 6.52^{**}$

Critical difference for the combination means at

a) 0.05 level 0.21

b) 0.01 level 0.28

Groups at 0.05 level: (G/7, G/MC, G/9, S/MC), (G/9, S/MC, S/7), (S/7, S/9)

Groups at 0.01 level: (G/7, G/MC, G/9, S/MC), (G/MC, G/9, S/MC, S/7), (S/7, S/9)

Table 4. Total nitrogen in apple leaves (percent dry weight) sampled June 25, 1957.

Replication	Combination					
	G/7	G/9	G/MC	S/7	S/9	S/MC
1	3.45	3.33	3.29	3.06	3.15	3.34
2	3.48	3.38	3.56	3.45	3.94	3.23
3	3.55	3.50	3.72	3.12	3.84	3.14
4	3.21	3.38	3.66	3.18	3.06	3.24
5	3.47	3.46	3.41	2.96	2.98	3.13
Mean	3.43	3.41	3.53	3.15	2.99	3.22

Calculated $F_{24}^{5} = 11.28^{**}$

Critical difference for the combination means at

a) 0.05 level 0.18

b) 0.01 level 0.24

Groups at 0.05 level: (G/MC, G/7, G/9), (S/MC), (S/7, S/9)

Groups at 0.01 level: (G/MC, G/7, G/9), (G/7, G/9, S/MC), (S/MC, S/7, S/9)

Table 5. Total nitrogen in apple leaves (percent dry weight) sampled July 16, 1957.

Replication	Combination					
	G/7	G/9	G/MC	S/7	S/9	S/mc
1	3.41	3.20	3.09	3.16	2.92	3.21
2	3.04	2.95	3.29	3.21	2.93	3.20
3	3.19	3.24	3.45	3.07	2.79	3.17
4	2.69	2.94	3.21	2.90	3.09	3.14
5	3.14	3.13	3.16	2.99	2.99	3.05
Mean	3.09	3.09	3.24	3.07	2.94	3.15

Calculated $F_{24}^{5} = 20.87$**

Critical difference for the combination means at

a) 0.05 level 0.20

b) 0.01 level 0.27

Groups at 0.05 level: (G/MC, S/MC, G/7, G/9, S/9),

(G/7, G/9, S/7, S/9)

Groups at 0.01 level: (G/MC, S/MC, G/7, G/9, S/7),

(G/MC, S/MC, G/7, G/9, S/7, S/9)

Table 6. Total nitrogen in apple leaves (percent dry weight) sampled August 6, 1957.

Replication	Combination					
	G/7	G/9	G/MC	S/7	S/9	S/MC
1	3.20	2.89	2.98	2.98	2.52	3.01
2	3.20	2.94	3.21	3.02	2.53	2.91
3	2.91	3.23	3.22	3.03	2.38	2.88
4	2.92	3.04	3.20	2.95	2.87	2.61
5	3.03	3.11	3.11	2.56	2.54	2.73
Mean	3.05	3.04	3.14	2.91	2.57	2.83

Calculated $F_{24}^{5} = 8.96^{**}$

Critical difference for the combination means at

a) 0.05 level 0.18

b) 0.01 level 0.25

Groups at 0.05 level: (G/MC, G/7, G/9), (G/7, G/9, S/7), (S/7, S/MC), (S/9)

Groups at 0.01 level: (G/MC, G/7, G/9, S/7), (G/7, G/9, S/7, S/MC), (S/9)

Table 7. Total nitrogen in apple leaves (percent dry weight) sampled August 27, 1957.

Replication	Combination					
	G/7	G/9	G/MC	S/7	S/9	S/MC
1	2.71	2.50	2.87	2.72	2.41	2.78
2	2.82	2.64	3.16	2.69	2.40	2.55
3	2.46	2.63	2.98	2.48	2.10	2.62
4	2.89	2.67	3.11	2.90	2.86	2.67
5	2.69	3.03	3.02	2.59	2.38	2.52
Mean	2.71	2.69	3.03	2.68	2.43	2.63

Calculated $F_{24}^{5} = 6.00^{**}$

Critical difference for the combination means at

a) 0.05 level 0.16

b) 0.01 level 0.22

Groups at 0.05 level: (G/MC), (G/7, G/9, S/7, S/MC), (S/9)

Groups at 0.01 level: (G/MC), (G/7, G/9, S/7, S/MC), (S/MC, S/9)

Table 8. Total nitrogen in apple leaves (percent dry weight) sampled September 17, 1957.

Replication	Combination					
	G/7	G/9	G/MC	S/7	S/9	S/MC
1	2.98	2.92	3.09	3.04	2.52	2.90
2	2.84	2.99	3.13	2.84	2.66	2.88
3	3.00	3.08	3.29	2.59	2.25	2.65
4	2.91	2.87	3.22	2.80	2.63	2.80
5	2.96	3.09	3.26	2.67	2.60	2.47
Mean	2.94	2.99	3.20	2.79	2.53	2.74

Calculated $F_{24}^{5} = 14.67^{**}$

Critical difference for the combination means at

a) 0.05 level 0.18

b) 0.01 level 0.24

Groups at 0.05 level: (G/MC), (G/9, G/7), (G/7, S/7), (S/7, S/MC), (S/9)

Groups at 0.01 level: (G/MC, G/9), (G/9, G/7, S/7), (G/7, S/7, S/MC), (S/9)

Table 9. Total nitrogen in apple leaves (percent dry weight) sampled October 8, 1957.

Replication	Combination					
	G/7	G/9	G/MC	S/7	S/9	S/MC
1	2.72	2.80	2.70	2.44	2.47	2.86
2	2.49	2.75	3.08	2.66	2.37	2.54
3	2.68	2.89	2.79	2.47	2.28	2.61
4	2.68	2.67	2.64	2.95	2.63	2.77
5	2.87	2.96	2.67	2.84	2.42	2.52
Mean	2.69	2.81	2.78	2.67	2.43	2.66

Calculated $F_{24}^{5} = 3.52$*

Critical difference for the combination means at

a) 0.05 level 0.21

Groups at 0.05 level: (G/9, G/MC, G/7, S/7, S/MC), (S/9)

Table 10. Seasonal average total nitrogen in apple leaves (percent dry weight) during the 1957 growth season.

Date	Combination						
	G/7	G/9	G/MC	S/7	S/9	S/MC	Mean
May 14	3.28	3.56	3.21	3.55	3.34	3.31	3.38
June 4	3.36	3.25	3.35	3.07	2.87	3.20	3.18
June 25	3.43	3.41	3.53	3.15	2.99	3.22	3.29
July 16	3.09	3.09	3.24	3.07	2.94	3.15	3.10
Aug. 6	3.05	3.04	3.14	2.91	2.57	2.83	2.92
Aug. 27	2.71	2.69	3.03	2.68	2.43	2.63	2.60
Sept. 17	2.94	2.99	3.20	2.79	2.53	2.74	2.86
Oct. 8	2.69	2.81	2.78	2.67	2.43	2.66	2.67
Mean	3.07	3.10	3.18	2.99	2.76	2.97	

Combination

Calculated $F_{35}^{5} = 13.68^{**}$

Critical difference for the combination means at

a) 0.05 level 0.14

b) 0.01 level 0.18

Groups at 0.05 level: (G/MC, G/9, G/7), (G/9, G/7, S/7, S/MC), (S/9)

Groups at 0.01 level: (G/MC, G/9, G/7), (G/9, G/7, S/7, S/MC), (S/9)

Date

Calculated $F_{35}^{7} = 33.52^{**}$

Critical difference for the date means at

a) 0.05 level 0.14

b) 0.01 level 0.18

Groups at 0.05 level: (May 14, June 25), (June 25, June 4), (June 4, July 16), (Aug. 6, Sept. 17), (Oct. 8, Aug. 27)

Groups at 0.01 level: (May 14, June 25), (June 25, June 4), (June 4, July 16), (Aug. 6, Sept. 17), (Oct. 8, Aug. 27)

Boynton and Compton (6) consider that the optimum balance for total nitrogen should be between 1.85 and 2.00 percent of the dry weight, and feel that fruit, color and quality, are seriously impaired when such samples contain more than 2.00 percent total nitrogen. In addition there is no increase in the yield. Conversely, if the value is lower than 1.85 percent, the yield is reduced although the quality may be good.

The calculated F value of the analysis of variance, for each sampling date show that the S/9 combination had always the lowest total nitrogen in the leaves all through the growing season except for the May 14 sample as seen in tables 2,3,4,5,6,7,8 and 9.

Table 10 shows clearly that at the beginning of the season, the average percent total nitrogen in the various combinations is significantly different from that at the other dates of sampling due to the large proportion of the meristematic tissue in the newly developing leaves which, with time, differentiates into the various tissues composing the leaf. There is much overlap between the combinations, the Golden Delicious being the higher variety as far as total nitrogen in the leaves is concerned.

Except for few Starking Delicious trees on the Malling understocks, none of the trees were bearing. One replicate of the S/9 combination, which had nine fruits, had a seasonal average of 2.57 percent total nitrogen. This value is low when compared to the other replicates in the same combination; the same applies, more or less, to the other combinations in which none of the replicates was bearing fruit.

The presence of flowers, and later fruits, has a highly significant effect upon the growth of the tree (62), and should be accounted for (29), due to the diversion and the use of the vital amino-acids, carbohydrates and mineral reserves in the leaves and tree, and to the early reproductive function and growth of the fruit (17,19,21,40,62). Thomas (48) found little difference between the composition of leaves from fruiting and non-fruiting shoots of apple trees and Emmert (14) secured results showing that "leaves from bearing trees were higher in nitrogen in all but three orchards".

When the yields are compared, although the data are inadequate, it seems that the more dwarfing understocks, Malling VII and Malling IX, have produced earlier bearing than the trees having Malus communis as a rootstock. This dwarfing, possibly due to its early bearing, is a result of some degree of uncongeniality (39), which is often desired. Malling IX fruited earlier and had more fruits than Malling VII; M. communis understock produced no fruit. This could be attributed to the ability of the Malling stocks to be more efficient utilizers of nitrogen found in the soil, a condition reported by Tkey and Brase (55). The Starking Delicious variety fruited earlier than the Golden Delicious when grown under identical conditions.

Irrespective of the variety, the understocks do not show any significant difference among themselves, as far as total nitrogen in the leaves is concerned (table 11). This result was also obtained for those understocks which have the Golden Delicious variety as the scion (table 12). But for those on which the Starking Delicious was grafted, the analysis of variance shows that the Malling IX was the lowest in total nitrogen while the other two stocks, Malling VII and M. communis, were statistically the same as seen in table 13.

The understocks, used in this experiment, produced trees having the same general appearance and size, which agrees with the view of Lincoln (23). The nonsignificant difference between them is shown in table 19.

The scions had a marked influence in determining the increase in

Table 11. Understock effect: seasonal average total nitrogen in Golden Delicious and Starking Delicious apple leaves (percent dry weight) sampled during the 1957 growth season.

Variety		Understock		
		Malling VII	Malling IX	*Malus communis*
Gold. Del.	1	3.15	3.04	3.12
	2	3.11	3.14	3.25
	3	3.01	3.14	3.25
	4	2.97	2.94	3.24
	5	3.10	3.26	3.06
Stark. Del.	1	3.06	2.78	3.08
	2	3.07	2.76	2.92
	3	2.92	2.57	2.94
	4	3.02	2.94	2.99
	5	2.86	2.78	2.90
Mean		3.03	2.94	3.08

Calculated $F_{27}^{2} = 2.12$

Table 12. Understock effect: seasonal average total nitrogen in Golden Delicious apple leaves (percent dry weight) sampled during the 1957 growth season.

Replication	Understock		
	Malling VII	Malling IX	*Malus communis*
1	3.15	3.04	3.12
2	3.11	3.14	3.25
3	3.01	3.14	3.25
4	2.97	2.94	3.24
5	3.10	3.26	3.06
Mean	3.07	3.10	3.18

Calculated $F_{12}^{2} = 2.00$

Table 13. Understock effect: seasonal average total nitrogen in Starking Delicious apple leaves (percent dry weight) sampled during the 1957 growth season.

Replication	Understock		
	Malling VII	Mallimg IX	*Malus communis*
1	3.06	2.78	3.08
2	3.07	2.76	2.92
3	2.92	2.57	2.94
4	3.02	2.94	2.99
5	2.86	2.78	2.90
Mean	2.99	2.77	2.97

Calculated $F_{12}^{2} = 7.40$**

Critical difference for the means at

a) 0.05 level 0.14

b) 0.01 level 0.20

Groups at 0.05 level: (Malling VII, *M. communis*), (Malling IX)

Groups at 0.01 level: (Malling VII, *M. communis*), (*M. communis*, Malling IX) the trunk circumference, in that the Starking Delicious variety made a significantly higher increase than the Golden Delicious as can be seen from tables 18 and 22. Besides, there was no difference between the understocks when used for the same variety. This agrees with the results obtained by Roberts (35) who says "a scion variety induces a root development which is typical for the variety", and therefore, these stocks absorb and translocate the nutrient from the soil the same way for that particular variety. But "there is a suggestion that the dwarfing stocks show a greater diameter than the standard stocks and Malling IX had the largest diameter", as reported by Shaw (40), but at this age ($2\frac{1}{2}$ years old), the effect of the dwarfing understocks on the trees is scarcely noticeable.

The analysis of variance for the varietal effect, irrespective of the rootstock, for the seasonal average total nitrogen in the Golden Delicious and Starking Delicious apple leaves, shows that the former variety had significantly higher total nitrogen than the latter variety as seen in table 14. Most probably this difference is varietal (19) as shown when the two varieties were grown on Malling IX and M. communis, as seen in tables 14,16 and 17, although the same effect was not evident on Malling VII (table 15).

Theoretically one should expect, on the basis of the data obtained in this study, that the Golden Delicious would always show a higher total nitrogen than the Starking Delicious, irrespective of the understock used; the one exception noted was Golden Delicious on Malling VII which may have been a stock-scion interaction. The results obtained show that the Golden Delicious variety did not influence the understocks (table 12), as far as the average total nitrogen in the leaves is concerned, while the Starking Delicious variety induced a statistical difference between Malling VII

Table 14. Varietal effect: seasonal average total nitrogen in Golden Delicious and Starking Delicious apple leaves (percent dry weight) on Malling VII, Malling IX and Malus communis understocks during the 1957 growth season.

Understock		Variety	
		Golden Delicious	Starking Delicious
Malling VII	1	3.15	3.06
	2	3.11	3.07
	3	3.01	2.92
	4	2.97	3.02
	5	3.10	2.86
Malling IX	1	3.04	2.78
	2	3.14	2.76
	3	3.14	2.57
	4	2.94	2.94
	5	3.26	2.78
M. communis	1	3.12	3.08
	2	3.25	2.92
	3	3.25	2.94
	4	3.24	2.99
	5	3.06	2.90
Mean		3.12	2.91

Calculated $F^{1}_{28} = 22.66^{**}$

Critical difference for the variety means at

a) 0.05 level 0.09

b) 0.01 level 0.12

Groups at 0.05 level: (Golden Delicious), (Starking Delicious)

Groups at 0.01 level: (Golden Delicious), (Starking Delicious)

Table 15. Varietal effect: seasonal average total nitrogen in apple leaves (percent dry weight) on Malling VII understock during the 1957 growth season.

Replication	Variety	
	Golden Delicious	Starking Delicious
1	3.15	3.06
2	3.11	3.07
3	3.01	2.92
4	2.97	3.02
5	3.10	2.86
Mean	3.07	2.99

Calculated $F\frac{1}{8} = 2.43$

Table 16. Varietal effect: seasonal average total nitrogen in apple leaves (percent dry weight) on Malling IX understock during the 1957 growth season.

Replication	Variety	
	Golden Delicious	Starking Delicious
1	3.04	2.78
2	3.14	2.76
3	3.14	2.57
4	2.94	2.94
5	3.26	2.78
Mean	3.10	2.75

Calculated $F_{8}^{1} = 378.50^{**}$

Critical difference for the variety means at

a) 0.05 level 0.18

b) 0.01 level 0.27

Groups at 0.05 level: (Golden Delicious), (Starking Delicious)

Groups at 0.01 level: (Golden Delicious), (Starking Delicious)

Table 17. Varietal effect: seasonal average total nitrogen in apple leaves (percent dry weight) on Malus communis understock during the 1957 growth season.

Replication	Variety	
	Golden Delicious	Starking Delicious
1	3.12	3.08
2	3.25	2.92
3	3.25	2.94
4	3.24	2.99
5	3.06	2.90
Mean	3.18	2.57

Calculated $F \frac{1}{8} = 19.83^{**}$

Critical difference for the variety means at

a) 0.05 level 0.11

b) 0.01 level 0.16

Groups at 0.05 level: (Golden Delicious), (Starking Delicious)

Groups at 0.01 level: (Golden Delicious), (Starking Delicious)

and M. communis (table 13). For the varietal effect, the data indicate that the influence of the Golden Delicious, on the understock, to induce a higher average seasonal total nitrogen in the leaves is still the same except for Malling VII which caused the same effect in both Golden Delicious and Starking Delicious varieties.

The increase in trunk circumference is as expected, except for one tree in the S/9 combination which made a 7.4 cm., an increase higher than the average value for this and the other combinations, as seen in table 18.

Tables 1 and 18 show that those combinations involving Golden Delicious, which had the significantly highest total nitrogen percentage in the leaves, made a significantly lower increase in their trunk circumference than the Starking Delicious variety.

The tables 18,19,20,21,22,23,24 and 25 on the increase in trunk circumference of the trees under study, at 30 cm. from the soil level, during the 1957 growth season indicate the absence of an interaction between the understocks and the varieties used, in that the understocks consistently induced statistically the same increase in the trunk circumference, irrespective of the variety. This was also true for both the Golden Delicious and the Starking Delicious varieties.

Irrigation did not cause an increase in the total nitrogen in the leaves, although the irrigation water contained pig urine. On the contrary, it seems that after irrigation the level of the nitrogen decreases possibly due to the filling of the soil air spaces with water, resulting in a poor respiration of the roots, thus decreasing their rate of absorption of the nutrient from the soil. When a sample was taken some 12 to 15 days after irrigation the soil moisture was at its optimum for root activity.

Table 18. Increase in trunk circumference of apple trees in centimeters (at 30 cm. from soil level) during the 1957 growth season.

Replication	Combination					
	G/7	G/9	G/MC	S/7	S/9	S/MC
1	4.2	5.1	3.5	4.1	5.2	4.3
2	2.5	3.4	4.6	6.5	5.0	5.3
3	3.2	3.2	4.1	6.1	4.6	5.5
4	3.3	4.1	3.3	5.1	7.4	5.4
5	3.2	3.2	3.7	5.3	4.7	5.2
Mean	3.3	3.8	3.8	5.4	5.4	5.1

Calculated $F_{24}^{5} = 7.12$**

Critical difference for the combination means at

a) 0.05 level 1.03

b) 0.01 level 1.40

Groups at 0.05 level: (S/7, S/9, S/MC), (G/9, G/MC, G/7)

Groups at 0.01 level: (S/7, S/9, S/MC), (S/MC, G/9, G/MC),

(G/9, G/MC, G/7)

Table 19. Understock effect: increase in trunk circumference of apple trees in centimeters (at 30 cm. from soil level) during the 1957 growth season.

Variety		Understock		
		Malling VII	Malling IX	*Malus communis*
Gold. Del.	1	4.2	5.1	3.5
	2	2.5	3.4	4.6
	3	3.2	3.2	4.1
	4	3.3	4.1	3.3
	5	3.2	3.2	3.7
Stark. Del.	1	4.1	5.2	4.3
	2	6.5	5.0	5.3
	3	6.1	4.6	5.5
	4	5.1	7.4	5.4
	5	5.3	4.7	5.2
Mean		4.4	4.6	4.5

Calculated $F_{27}^{2} = 0.11$

Table 20. Understock effect: increase in trunk circumference of Golden Delicious apple trees in centimeters (at 30 cm. from soil level) during the 1957 growth season.

Replication	Understock		
	Malling VII	Malling IX	*Malus communis*
1	4.2	5.1	3.5
2	2.5	3.4	4.6
3	3.2	3.2	4.1
4	3.3	4.1	3.3
5	3.2	3.2	3.7
Mean	3.3	3.8	3.8

Calculated $F_{27}^{2} = 2.54$

Table 21. Understock effect: increase in trunk circumference of Starking Delicious apple trees in centimeters (at 30 cm. from soil level) during the 1957 growth season.

Replication	Understock		
	Malling VII	Malling IX	*Malus communis*
1	4.1	5.2	4.3
2	6.5	5.0	5.3
3	6.1	4.6	5.5
4	5.1	7.4	5.4
5	5.3	4.7	5.2
Mean	5.4	5.4	5.1

Calculated $F_{27}^{2} = 0.32$

Table 22. Varietal effect: increase in trunk circumference of apple trees in centimeters (at 30 cm. from soil level) during the 1957 growth season.

Understock		Variety	
		Golden Delicious	Starking Delicious
Malling VII	1	4.2	4.1
	2	2.5	6.5
	3	3.2	6.1
	4	3.3	5.1
	5	3.2	5.3
Malling IX	1	5.1	5.2
	2	3.4	5.0
	3	3.2	4.6
	4	4.1	7.4
	5	3.2	4.7
M. communis	1	3.5	4.3
	2	4.6	5.3
	3	4.1	5.5
	4	3.3	5.4
	5	3.7	5.2
Mean		3.6	5.3

Calculated $F^{1}_{28} = 36.46^{**}$

Critical difference for the variety means at

a) 0.05 level 0.4

b) 0.01 level 0.5

Groups at 0.05 level: (Starking Delicious), (Golden Delicious)

Groups at 0.01 level: (Starking Delicious), (Golden Delicious)

Table 23. Varietal effect: increase in trunk circumference of apple trees in centimeters (at 30 cm. from soil level) on Malling VII understock during the 1957 growth season.

Replication	Variety	
	Golden Delicious	Starking Delicious
1	4.2	4.1
2	2.5	6.5
3	3.2	6.1
4	3.3	5.1
5	3.2	5.3
Mean	3.3	5.4

Calculated $F \frac{1}{8} = 18.49^{**}$

Critical difference for the variety means at

a) 0.05 level 1.2

b) 0.01 level 1.7

Groups at 0.05 level: (Starking Delicious), (Golden Delicious)

Groups at 0.01 level: (Starking Delicious), (Golden Delicious)

Table 24. Varietal effect: increase in trunk circumference of apple trees in centimeters (at 30 cm. from soil level) on Malling IX understock during the 1957 growth season.

Replication	Variety	
	Golden Delicious	Starking Delicious
1	5.1	5.2
2	3.4	5.0
3	3.2	4.6
4	4.1	7.4
5	3.2	4.7
Mean	3.8	5.4

Calculated $F_{8}^{1} = 6.25^{**}$

Critical difference for the variety means at

a) 0.05 level 0.9

b) 0.01 level 1.3

Groups at 0.05 level: (Starking Delicious), (Golden Delicious)

Groups at 0.01 level: (Starking Delicious), (Golden Delicious)

Table 25. Varietal effect: increase in trunk circumference of apple trees in centimeters (at 30 cm. from soil level) on *Malus communis* understock during the 1957 growth season.

Replication	Variety	
	Golden Delicious	Starking Delicious
1	3.5	4.3
2	4.6	5.3
3	4.1	5.5
4	3.3	5.4
5	3.7	5.2
Mean	3.8	5.1

Calculated $F\frac{1}{8} = 16.90^{**}$

Critical difference for the variety means at

a) 0.05 level 0.2

b) 0.01 level 0.3

Groups at 0.05 level: (Starking Delicious), (Golden Delicious)

Groups at 0.01 level: (Starking Delicious), (Golden Delicious)

The coefficient of correlation between the total nitrogen in the leaves and the increase in the trunk circumference was calculated using the formula:

$$r = \frac{n\sum xy - (\Sigma x)(\Sigma y)}{\sqrt{\left[n\Sigma x_i^2 - (\Sigma x_i)^2\right]\left[n\Sigma y_i^2 - (\Sigma y_i)^2\right]}}$$

where

n is the number of the trees

x is the increase in the trunk circumference for each tree (centimeters)

y is the average total nitrogen in the leaves (percent dry weight)

$$r = - 0.44^*$$

From this calculation, it can be concluded that the estimate of the correlation coefficient is negative, i.e., as the trunk circumference increases, the percent total nitrogen in the leaves decreases during the growth season, and it is significantly different from zero at the 5 percent level.

Summary and Conclusions

Three understocks, Malling VII, Malling IX and Malus communis, and two apple varieties, Golden Delicious and Starking Delicious, as scions, constituted the six different combinations used for study in an orchard in Khonsharah region (Mount Lebanon). Each combination had five replicates.

The statistical analysis of the results of this study seem to indicate the following:

I. As far as the total nitrogen in the leaves is concerned:

a. It decreased significantly with the advance of the season.

b. It was different in the various combinations at the separate dates of sampling.

c. The different understocks did not influence the amount except when Starking Delicious was the scion on Malling IX.

d. It was higher in the Golden Delicious as compared with the Starking Delicious, except when Malling VII was the understock resulting in the two varieties being the same.

e. It was the lowest in the S/9 combination throughout the growing season except for May 14 where all the combinations were statistically the same.

II. As far as the increase in the trunk circumference is concerned:

a. It was different in the various combinations, the Starking Delicious making a greater increase than the Golden Delicious.

b. It was the same in the different understocks, and not influenced by the scion variety.

The coefficient of correlation between the total nitrogen in the leaves and the increase in the trunk circumference is negative and significantly different from zero at the 0.05 level.

With the results obtained in this study, it would be futile to set up standards for "critical values" or "optimmm values", because a one-year study is not enough from which to draw definite conclusions; the data show a trend, which might be accepted or rejected if the investigation were to be continued for several years.

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