Investigation of variations in the composition of the Timothy Plant (Phleum Pratense)

A THESIS

by

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INTRODUCTION

The cultivation of the Timothy plant as a forage crop has been practised in various countries particularly in Canada and the United States of America for a considerable length of time. As such, a knowledge of the variation in the composition of the plant at different periods of growth and maturity with a view to reflect the nutrient status of the plant and to indicate the levels of nutrient components at which effects due to deficiencies and excesses may be expected becomes important. In application of methods for diagnostic purposes arbitrary threshold values for deficiencies should be assigned for individual elements or components of the plant, levels associated with the onset of visual signs of particular deficiencies. To find these values it is incumbent to correlate them with the seasonal trends of fluctuations of the different components of the plant.

For a long time agricultural scientists have endeavoured to evaluate soils for purposes of growing crop and to determine fertilizer needs for maximum crop output by chemical analysis of soil. More recently, attention has been directed to establishment of "critical levels" for the different plant components in the different parts of the plant to evaluate arbitrary values for ideal growth in as much as the nutritive value is concerned. Attempts towards this end have been met with but limited success in standardising the methods for assessing crop growth against field performances. The failures have been due to various reasons among which (1) unsatisfactory sampling, (2) the inevitable difficulty of properly assessing all the factors which affect the plant growth and (3) the environmental conditions which vary from place to place, may be emphasized.

The extensive use of the 'visual diagnosis' method for the identification of deficiencies has shown necessity for developing confirmatory field tests for some crops where symptoms may not be well marked and for other crops where they may be masked by damage caused by insects, fungus diseases and presence of certain mineral elements in excess. Whether or not these visual diagnoses and confirmatory field tests could be avoided by only chemical analysis of the tissue samples from the plant remain yet to be explored.

Standards for the critical concentrations of mineral nutrients in different plants have already been fixed by various workers and these standards have been found to be independent of the soil type. But these standards do not solve the problem of overall necessity of the knowledge on the nutritive status of the plant, for its efficient use unless definite correlations are established between the mineral constituents and the other components of the plant.

A knowledge on the composition of the plant including the variations therein due to the interrelationships between nutrients thus becomes essential. With a view to gain an overall picture of the nutritive status of the timothy plant attempts have been made to investigate the variations in the composition by chemical analysis at different periods of maturity.

REVIEW OF LITERATURE

In a study on the ash content of plants, de Saussure carried out plant tissue analysis early in 1804. He showed that the amount of nutrient varied with the species, the plant part involved, the stage of development and the soil on which the plant grew.

In 1840 Liebig (52) suggested the regulation of fertiliser application to the soil on the basis of plant ash analysis. He considered the percentage nutrient content of plants as constant and the nutrient composition, therefore, as representing their nutrient needs. His idea though developed from the determination of a few of the elements, was partially true; but since then more elaborate suggestions and theories have been put forward.

Weinhold (118) suggested that plants growing most abundantly in one location were well adapted to the nutrients available in that soil. So ash analysis would provide information on the relative status of the assimilable nutrients present. This is an important diagnostic approach to plant analysis. Although Weinhold failed to establish his idea, Hellriegel (36) growing barley in sand culture found that the potassium content in straw and grain increased with increasing supply. Consequently, he proposed that the analysis of crop plants might provide an index of nutrient availability in the soil. Similar types of suggestions were put forward by a great number of workers for the following sixty years.

Goodall and Gregory (29) report: "Heinrich suggested a specific plant part might be more appropriate than a whole grass plant sample. Analyzing oat roots he proposed his 'Law of Minimum' a level to which an element in low supply would go into the root. (Liebig also suggested similar terms but in different sense). This was amended by Von Dikow who suggested a 'Law of Maximum', the concentration of an element in the root above which increasing supply would not have increased plant growth. Stahl-Schroder rejected this 'Law of Maximum' because he found evidence of luxury consumption. Both he and Helmkampf doubted the advisability of selecting roots for analysis. It remained for Atterberg of Sweden to continue the work using the aerial portions of oats. He attempted to set up optimum ratio for the inorganic constituents for yield of grain and tissue. This established a trend for subsequent work during the late 19th century. In 1889 Von Seelhort carried out a fertiliser experiment on twenty-four different soils in pot cultures. The plant composition was correlated to some degree with yield responses due to fertiliser treatments. He emphasized that the correlation of concentration of one nutrient alone was not sufficient to predict a response. Differences of opinion were wide among workers of that time as to the plant part most suitable for diagnostic techniques."

Lawes and Gilbert (51) found that plants may need in the fertilizer more phosphorus than other nutrients although they contain less phosphorous than many other nutrients; so they concluded that the nutrient composition of the crop is no direct guide to fertilizer need. Wolff (119) using increments of nutrients in water cultures reported the minimum requirements of the mature oat plants for a single nutrient in terms of their percentage content in the plant. His minimum percentage is really the threshold optimum and corresponds to the critical percentage as used by Macy (57).

In 1905 Hall (32) suggested that plant analysis might not be more useful than soil analysis because of great variations in the analytical data. As a means of overcoming the variations, he suggested that some plant or weed fairly common to all soils be chosen as an indicator plant. His results showed that practical use of plant analysis was not yet possible. Hall further considered the idea of a critical proportion of each nutrient being needed in the ash of plants, but he concluded that while the supply of a nutrient did effect its percentage content in the plant, the effect of other factors was as great or greater. This was the conclusion of almost all the later investigators in this field.

Ulrich (110) points out that between 1905 and 1919 little serious work was carried out on plant tissue analysis. In 1919 Pfeiffer et al (78) published results of investigation on dried plant tissue. They proposed that there is a definite relationship between the percentage content of a nutrient in a plant and its sufficiency as follows: For those points on the yield curves at which the increase divided by the current yield (the subtangent) lead to the same value, the crop concerned shows the same percentage nutrient content. They compared nutrient contents with yields rather than with yield responses, and they failed to designate the needed percentage of any nutrient. Their statement of the law seems to be complicated, it may more simply be stated that 'the sufficiency of a nutrient is a function of its percentage content in the plant'. The same theory as stated by Macy may be quoted as follows: "There is a critical percentage of each nutrient in each kind of plant, above which there is a luxury consumption and below which there is a poverty adjustment which is almost proportional to the deficiency until a minimum percentage is reached".

In 1927 Gilbert and Hardin (27) undertook analysis of fresh plant material in America. From these beginnings followed two modes of investigations and methods of interpreting results. One avenue of research was the investigation of dried or preserved plant tissues by means of chemical analyses, accounting for the overall chemical composition of the material. The second approach led to the study of fewer tissue components in fresh or living plant parts by means of rapid tests.

It was not until recently that the necessity of plant analysis as a measure of plant nutrition was felt intensely and as such a great number of works were published by many European workers on the possibilities of plant analysis. Goodall and Gregory (29) point out that the expansion of research owed much to the subsequent change in emphasis in this field. Less attention is being given to the interpretation of results in terms of soil fertility and more in terms of nutritional status of the plant. But much of the early works were concerned with roots, whole plants or senile parts as opposed to the present trend of analysing actively functioning parts; apparently some degree of success was obtained by the early workers in the accuracy of their forecasts regarding fertilizer needs. Plant physiologists operating in such applied fields of investigation as agronomy, horticulture and pathology have frequently experienced need for examination of their problems and results in terms of chemical composition of tissues. In many cases, however, only the comparative results are concerned and clearly no definite recommendation towards guarantee of infallibility of those results can be made with such data. A reasonable exercise of critical faculties is invariably demanded of those who would attempt the execution of chemical analysis.

Recently, the major objectives of plant analysis as outlined by Ulrich (110) have been to predict the nutrient requirements of crops on the basis of a single sample taken in midseason, and to determine the nutrient status of the crop at that time. This involves some study of yield response at different levels of a limiting nutrient correlated with the per cent of this element in plant tissue.

Liebig (52) proposed a 'Law of the Minimum' which states that the growth of the plant is governed by the nutrient which is in minimum supply. He envisaged the relationship between the yield and the amount of nutrient supplied as linear. Mitscherlich (63) propounded a different 'Law of the Minimum', a sort of diminishing return relationship, in which a yield response inresponse to an increased nutrient supply, will depend on the level of the nutrient already present. He states that the increase in yield per unit of limiting nutrient applied is directly proportional to the decrement from the maximum yield.

In 1936 Macy (57) proposed his theory of 'critical percentage' which has already been stated. He showed that the theory was comparable with the results of the earlier workers. That is, in the minimum percentage portion of the curve when the level of nutrient in the plant is constant Liebig's law holds. During the 'poverty adjustment' when the slope of the curve begins to rise so that response to the supply of nutrient decreases progressively, Mitscherlich's law holds. When the 'critical percentage' or level of sufficiency is reached luxury consumption sets in and Liebig's law holds again. It is in this zone of transition between the 'minimum percentage' and 'luxury consumption' that many crops fall in practice.

The critical concentration or percentage concept as initiated by Macy, which justified the search for critical levels as reported by Tyner (109), Lundegardh (53), Hill and Cannon (38) and many others (3, 42, 50, 94, 102), provides the basis for the common usage of the term 'deficiency' as defined by Goodall and Gregory. Insufficient work has actually been done on the critical nutrition concept (Ulrich), but a number of workers have indicated that the critical percentage is constant regardless of other factors even with a wide range of varying environmental factors (Goodall and Gregory). Thus the critical percentage for potassium or other elements may be lowered by the presence of Sodium or similar constituent. Lundegardh claims that leaf content is an integrated result of all factors and is quite irrelevant of environment. But it will appear, however, that there is insufficient evidence to extend indiscriminately the use of critical values for nutrient levels in a given crop as determined under one particular set of conditions. Further the concept assumed that the increase in yield of any crop is obtained only on correcting the established deficiency. This limits the practical application of the concept because very little information may be obtained regarding the potential supply of nutrients other than that which is deficient.

An alternative suggestion on the plant nutrition studies was the concept of nutrient balance within the plant originally introduced by Lagatu and Maume (47), and Thomas (103). The concept propounds a hypothesis of dependence of plant growth on the proper balance of nutrients (N, P and K), as well as on their intensity, and that the variations are reflected in the composition of the leaf. As a matter of fact Thomas advanced a criticism of the concepts of Liebig and others whom, he said, assessed plant nutrition in a quantitative sense only. In addition to the amount of the elements present in a leaf at any given time, he maintained that the ratio or physiological relation between the elements was also an important expression of the adequacy of plant nutrition. From corn data he showed that different treatments could change the ratio between the elements without changing the intensity of nutrition. This idea has been modified by many workers to adopt it to a wider interpretation and to include certain other elements in various crops.

It will appear from the previous discussion that plant analysis can be utilised for diagnosis of nutrient requirements of crops but sufficient work must be done to standardise such approach before practical application of the principle. Starting from the early twenties of the twentieth century, agricultural scientists had been trying to standardise one such method to meet all objections, and research on plant analysis as a tool for assessing the status of plant nutrition has been greatly intensified during the last quarter of the century. Much of this work has been in the development of 'rapid tissue tests' for the determination of current nutrient requirements for general advisory work. These tests have usually been carried out on green tissue or expressed sap, the theory being that the concentration of nutrients in the conductive tissues and solutions obtained therefrom represents the current rate of nutrient uptake. 'Rapid tissue tests' for the estimation of the nutrient status of plants has its origin in the United States of America. Early work was done by Hoffer (39), who made tests for iron and potassium in fresh corn stalk (Zea Mais) and showed that when potassium was deficient, iron accumulated in the nodal tissues. The value of rapid tests made in the field (as by Hoffer) is discounted by the high degree of plant-to-plant variability. MacGillivray et al (59) using thin sections of leaf petioles of tomatoes immersed in platinic chloride solution for twenty-four hours made microscopic studies of the relative abundance of potassium platinic chloride crystals. This was used as an indication of the potassium status of the plant. A later development was the study of the nutrient content of expressed sap by Gilbert and Hardin (27) for N, P and K by grinding fresh material and straining through muslin. McCool & Weldon (55) used a similar method for K and P using sap extracted at pressure ½ to 1 ton per square inch. Variations in the composition of extracted sap have been found by Knudson and Ginsberg (45) and Gassner & Goeze (25, 26), especially when the pressure used to obtain it is varied or by time difference in attaining the same pressure. Work along this line has not yielded consistent results and not many publications appeared during those years. At a later date a number of workers have used a variety of solvents for the extraction of soluble nutrients from plants. The simplest procedure is the extraction with cold water as used by Nightingale (73, 74, 75) for the detection of calcium deficiency in sugarcane and pineapple. Burkhart and Page (12) used boiling water for the determination of N, P and K deficiencies in peanut and cotton. The use of an organic solvent, 2% acetic acid, was introduced by Emmert (19, 20) for detecting nitrate N, P and K, and Carolus (13, 14) extended the scheme to include calcium and magnesium in vegetable crops.

The extraction of nutrients by the Purdue technique devised by Thornton et al(104,105) is made by chemical reagents used for detecting the required nutrients viz. cobaltinitrite for K, ammonium molybdate for P, and diphenylamine in sulphuric acid for nitrate N. Tests which have been made on maize and cotton are only roughly quantitative because of the fact that the tissue is in situ and nutrients continue to diffuse into solution. Carolus (13) and Hester (35) having used the waring blender for the extraction of nutrients from fresh tissues, the former used 2% acetic acid, whereas Hester used acetate buffer solution as developed by Morgan (64) for the latter's universal soil testing system. These methods, however, have the disadvantage of not being usable in the field.

A series of papers have been published from Long Ashton Agricultural and Horticultural Research Station (68, 72, 83) on the development of tissue test techniques. Chemical tissue tests have been carried out on fresh plant material with known deficiencies or grown with known nutrient treatment. The results obtained suggest that the method will be valuable for diagnostic purposes, although further experience will be necessary to enable standards to be fixed for various types of plants. Nicholas and Jones (69, 70, 71) at Long Ashton have shown that the tissue test method was useful in the diagnosis of mineral nutrient deficiencies in plants. The correlation between this method, manurial treatments and visual diagnosis was good. There was agreement between the chemical data and tissue test values; both methods usually showed the nutrient condition of plants. Their results suggested that the tissue test technique would be valuable for diagnostic purposes and that the problems regarding (1), the best time for sampling the plant material, (2) the effect of interfering substances on the extraction of cations and anions from tissues and (3) the fixing of nutrient standard for various types of plants would have to be solved.

It appears from the previous discussions that tissue tests if suitably organised can very fruitfully be utilised in studying the nutritive status of a plant. It will further be observed that no complete technique of tissue testing has yet been evolved.

It is evident that no one method will be adequate for assessing the nutritive status of a plant. It appears suggestive that in the study of nutrition of a plant the method of sam pling is more important than the technique of chemical snalysis: The samples should be collected at definite periods of growth and analysed by different appropriate methods for chemical constituents. In this way more insight into the nature of variation of chemical constituents as effected by the environmental conditions and internal correlations at different stages of growth will be achieved.

It has been found that most of the earlier workers analysed the whole plant for diagnostic purposes. Analyses of the whole plant at different periods would give only the variations at different periods of maturity, but the variations are not due to the environmental condition only; translocation and interaction between constituents are no less contributory to these variations. Such data on the chemical analysis of the whole plant may lead to erroneous diagnostic assumptions. Analyses of the more actively functioning parts such as internodes of the plant, will perhaps throw more light on the study of the variations in the nutritive constituents of the plant.

In the investigation of variations in the composition of the timothy plant as an attempt towards the successful assessment of the nutritive status, chemical tissue tests with particular reference to more actively functioning parts, such as internodes and the storage organs, therefore, become very important.

Early studies on the variations of chemical composition of the timothy plant were made by Haigh (31). He separated the plant into heads, stalks, leaves, stubble and roots and subjected them to chemical analysis for the usual feeding stuff constituents besides potassium and phosphorus in the ash and found that timothy takes up nitrogen and increases in ash constituents most rapidly in the young stages; the dry matter in the roots increased but fiber remained constant, and the other constituents pass out of the roots into the growing plant above ground during the ripening period of heads. His study though incomplete in various ways was one of the fifst steps towards the assessment of the composition of the timothy plant.

Waters (117) studied the influence of maturity upon yield and composition of timothy hay. He made a study of cuttings beginning June 12, when the plants were in full head, also several other subsequent dates. The second cutting when the plants were in full bloom gave the largest yield of digestible dry matter, protein, fat, crude fiber and nitrogen free extract. During the same period Trowbridge et al (106, 107) studied the changes in the chemical composition of the timothy plants during growth and ripening with a comparative study of the wheat plant. They found that both plants took up nitrogenous and ash constituents and contained larger amounts of water during the early stages of growth. In both plants the per cent protein, ash and ether extract showed a tendency to reach a higher value in the young than in the mature plant. The per cent of crude fiber and nitrogen-free extract was greater at maturity. Similar results were reported by Fagan (22) also.

Nehring (66) studied the influence of the soil reaction and fertilizing on the composition in pot tests with timothy and red clover. He found that besides an influence of the reaction on the yields, there was a change in the composition especially in relation to the content of crude protein and of different mineral constituents, (CaO, P_2O_5) which increased distinctly under the influence of lime additions. Field tests also showed similar results. Additions of lime effected a considerable increase in yield and a distinct change in composition. As to crude protein, the effect of lime was not constant.

Evans (21) conducted a ten year test using $NaNO_3$, superphosphate and KCl on timothy meadow and found that crop increases resulted for the fertilizer addition; greatest increases were noted with $NaNO_3$, followed by superphosphate. Tincker (101) conducted experiments on the effect of day-length upon the growth and chemical composition of the tissues of certain economic plants, timothy being one of them. His studies were concerned with the alteration of character and amount of growth of various plants with altered length of day, both throughout the season and at various stages in their growth.

Different parts of the plant were analysed and at various periods. He has shown that the length of day factor governs the utilisation of the carbohydrate manufactured. He analysed for crude protein, ash and carbohydrate.

Smirnova (95) studied the biochemistry of the timothy plant. He found the greatest variation in the contents of protein.

A study over a seven-year-period was made by Archibold et al (1) of the chemical composition of certain grasses (one being timothy) and legumes grown as practically pure stands. Within the same species differences were noted in the several constituents of the aerial part of the plant except in nitrogen and phosphorus; the greatest variations were observed in the magnesium content.

Phillips et al (79, 82) determined the composition of the tops and roots of the timothy plant at successive stages of growth. They found that the percentage nitrogen in the tops and roots of the plant decreased generally as the plants matured, while the quantity of nitrogen in the tops increased. The quantity of nitrogen in the roots showed little variation in the early stages of growth but in later stages the increase was more pronounced.

Phillips and Smith (80) studied the variation in the composition of the timothy plant with particular emphasis on the carbohydrates of the plant because of their important bearing on the physiological functions of the plant and on animal nutrition.

Soluble solids, glucose, fructose, sucrose, total sugars, fructosan produced by hydrodysis with 0.2 N and with N H_2SO_4 and the non-fermentable part produced by treatment with taka-diastase,cellulose, lignin and soluble, insoluble and protein nitrogen based on dry weight and total solids based on green weight were determined by them. The sugar contents of successive cuttings of timothy decreased markedly while lignin and cellulose increased. Lignin and non-fermentable reducing substances liberated by N H_2SO_4 were especially high in the heads of mature plants. More cellulose was contained in the stalks than in the heads. Fructosan accumulated in the lower sections of stalks of the plants nearing maturity. It was, however, a minor constituent of the samples.

Harper and Phillips (34) analysed the storage organs to determine what changes occur in the sugar and fructosan content during a growing season and on over-wintering. They found that until the period of blooming fructosan accumulated rapidly, then it remained unchanged. From the development of heads to cutting or during the mid-summer formation of new shoots, no decrease occurred. Fructosan began to decrease in October; when growth started the following May, a pronounced decrease occurred. To account for the high glucose and low fructosan and the reverse of this during its utilisation, they suggested a scheme based on the mutorotation of glucose and fructose and their conversion into sucrose and fructosan.

Robinson (89) studied the response of various forage grasses including timothy to phosphate fertilization under greenhouse conditions. Timothy showed increases in yields of dry matter and percentage increases in yields from high rates of phosphate fertilization. He showed that the differences in phosphate response could not be attributed to a deficiency of nutrients other than phosphorus. Similar fertilizer reponses by timothy have been demonstrated by Yoder (123), Bizzell and Leland (7) and many others.

Svanberg and Ekman (99) determined the magnesium content in timothy grown on Swedish soil. They found that the usual variation between different samples was high. On the other-hand, Keranen (44) showed that hay harvested at the beginning of the timothy blooming period showed uniformity and had a higher mineral content than that harvested later.

Price et al (85) also determined the mineral constituents and showed that under different soil and climatic conditions in Virginia differences in mineral composition existed between the same species grown under different conditions. Beeson et al (5) analysed the first cuttings of timothy and other grasses and found that there was an overall correlation of composition with yield. In 1949 Sarova (91) determined the chemical composition (N, P, K and Ca) of timothy and showed that timothy is one of the forage crops which contain a very low content of the minerals in comparison to the other crops.

Scharrer (92) showed that the timely use of nitrogen fertilizer in growing timothy is important. The protein yield and protein content are increased by extremely high and proportionately late nitrogen application. He, however, suggests that proper weather conditions are essential for success. Completely contradictory results have been obtained by Brown and Munsell (11). They showed that timothy yields less dry matter and protein as a result of fertilization with nitrogen.

Hvidsten et al (41) in Europe studied the composition of timothy plant at various stages of growth. Content of total organic matter was not greatly affected by the type of season or the date of cutting whereas both crude and pure protein were higher in a warm dry season than in a cool wet one.

Odelien (76) made a preliminary study with heavy applications of artificial fertilizer on timothy. Chemical analysis of timothy demonstrated the increase in crude nitrogen and ash with increasing amounts of fertilizer. His later experiments showed increases in saccharose content of the hay. More systematic study on the water soluble carbohydrate content during the normal life cycle of timothy has been made by Waite and Boyd (113, 114). They analysed leaf, stem and head and found that total free hexose content was low in all fractions (in the whole plant above ground) throughout the test period. The sucrose content of stem and of leaf of timothy were similar and rose to a maximum during May - June. Fructosan was the major carbohydrate in all of the samples and showed two maxima, one in May and the other in July. Richards and

Reid (88) also attempted to study the composition of the timothy plant. They differentiated three growth stages of timothy - vegetative, boot-to-early head, and full bloom. At successive stages of growth the amounts of cell wall constituents (lignin, cellulose, hemi-cellulose) increased progressively and those of pectins, sugars, crude protein and ether extract decreased.

Waite and Sastri (115) analysed the timothy plant to study the composition during seasonal growth and found that the ratio of leaf to stem fell sharply during the growing season, with corresponding increase in contents of crude fiber and decrease in protein, carotene and ether soluble matter. They found a negative correlation between protein and fiber.

Waite and Boyd (114) determined the crude protein, water soluble carbohydrate including glucose, fructose, sucrose and fructosan of timothy. They showed that timothy grown in spring and cut before mid-June, which had florally developing growing points and low stem leaf ratio had low protein and high soluble carbohydrate contents. The relation reversed later in the more mature period of growth.

One of the latest studies on the composition of the timothy plant is that of Homb (40) who analysed the hay for calcium, potassium, protein, crude fiber, ash, lignin, ether extract, dry matter, true protein, amino acid, nitrogen, sugar, nitrogen-free extract and carotene. He showed that dry matter, crude fiber, and lignin increased while the rest decreased with age. He analysed the whole plant.

It is evident that although a great number of studies have been made on the composition of timothy, apparently contradictory or supporting the results of one another, no complete and satisfactory technique has yet been established to assess the nutritive status of the plant at any one time.

EXPERIMENTAL MATERIALS & METHODS

DESCRIPTION OF THE AREA

The soil samples and the plant materials used in this study were collected from one of the fields operated by the Agronomy Department at Macdonald College, P.Q. The actual experimental area is designated 'Range 30, East field'. The condition of growth of the timothy crop produced on this area is similar to that found on farms in the neighbouring region, namely, the St. Lawrence Plain of Southern Quebec. Two soil series were involved - Chateauguay Clay Loam and Macdonald Clay Loam, the experimental area on the former soil being situated east of that on the latter. Both soils were formed on alluvial material and both overlie calcareous till. The Chateauguay has been classified as a weakly developed gray-brown podsolic soil and the Macdonald soil as belonging to the gray gleizolic group. The Macdonald clay loam is somewhat imperfectly drained whereas the Chateauguay soil has moderately good drainage. The two are found in association, the Chateauguay on undulating, the Macdonald on level to slightly depressed relief. These soils have been described by Lajoie and Baril (49). The complete description of the soils has been given in Appendix A.

The area was cropped to oats and timothy, timothy being continuous for a considerable period of time. The cropping history of the area from 1943 - 1954 has been tabulated in Table I.

A soil map of the area has been included to show the exact site of the plots and division of the main plots into sub-plots, during 1953. and 1954. In 1953, the first year of this investigation, two

TABLE I

CROPPING HISTORY OF THE AREA

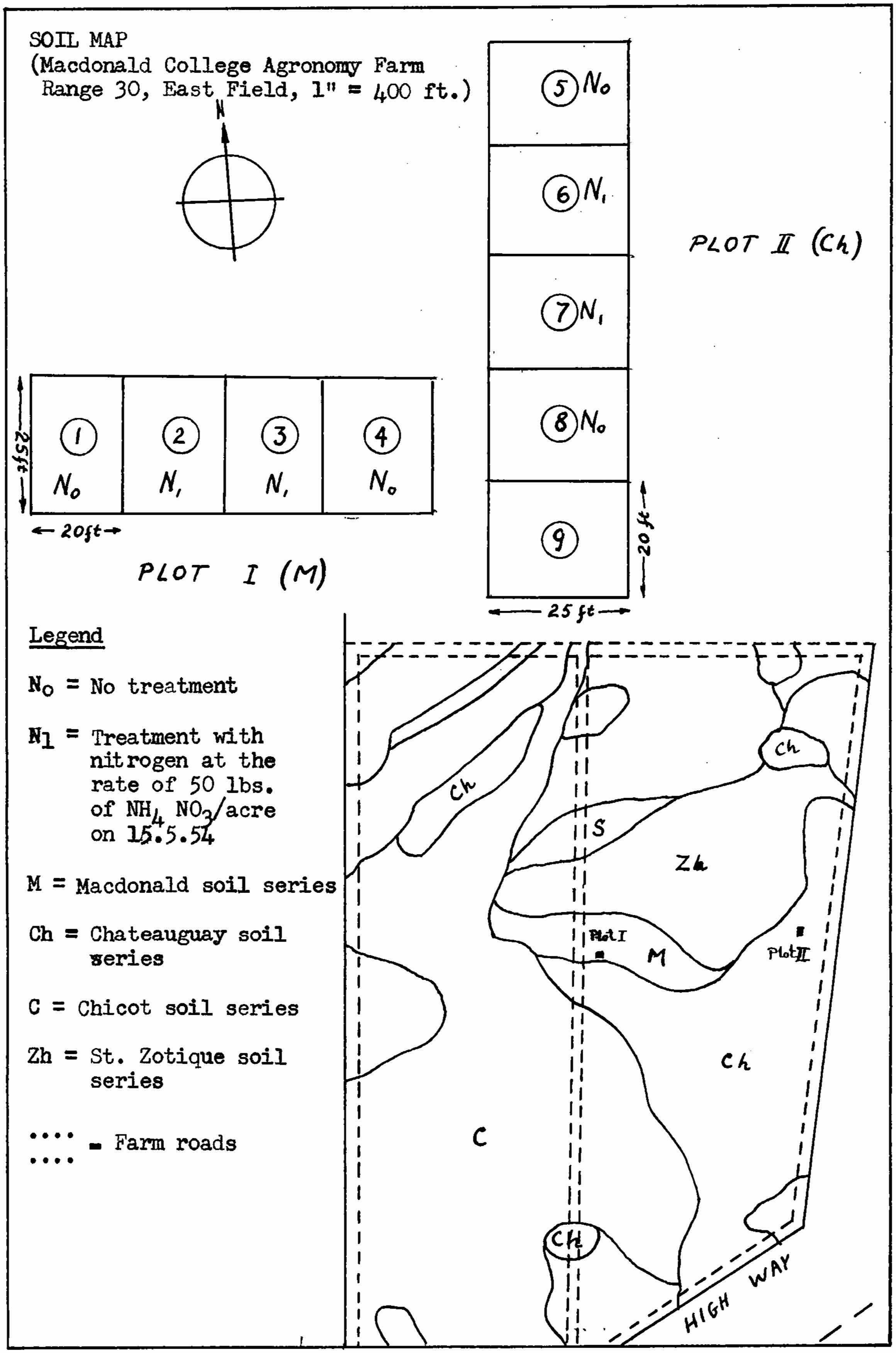
RANGE 30, EAST FIELD#

MACDONALD COLLEGE.

Period	Crop grown	Fertiliser applied
1943-1947	Timothy only	Nitrogenous fertiliser as $NaNO_3$ only in 1945
1948-1949	Oats, timothy being seeded with the second oat crop	2-12-10 fertiliser at seeding at the rate of 300 lbs./acre
1950-1954	Timothy only	No fertilizer

#By courtesy, Department of Agronomy, Macdonald College.

plots marked I (from Macdonald soil) and II (from Chateauguay soil) were chosen from this area as shown on the soil map. Plot I was divided into four sub-plots measuring 25' x 20' and Plot II to five such plots, as shown on the enlarged map, and samples for analytical work were collected from these sub-plots.


SOIL MAP
(Macdonald College Agronomy Farm
Range 30, East Field, 1" = 400 ft.)
N
5 No
6 N1
7 N1
8 No
9
PLOT II (Ch)
1 No
2 N1
3 N1
4 No
25 ft
20 ft
PLOT I (M)
20 ft
25 ft
Legend
N_0 = No treatment
N_1 = Treatment with nitrogen at the rate of 50 lbs. of NH_4 NO_3/acre on 15.5.54
M = Macdonald soil series
Ch = Chateauguay soil series
C = Chicot soil series
Zh = St. Zotique soil series
.... = Farm roads
Ch
Ch
S
Zh
Plot I
M
Plot II
Ch
C
Ch
HIGH WAY

SAMPLING

IST YEAR (1953). In this investigation composite samples representative of different sub-plots were collected at different stages of growth of the plant. For the stem-leaf samples the second internode including the second node and a leaf was collected in 1953. This part has been assumed to represent both a conducting tissue and a leaf at approximate maturity between the storage and reproductive part of the plant. The importance of morphologically similar parts of the plant for comparison has been stressed in view of the existence of a nutrient gradient within the plant. With a view to compensate for the diurnal fluctuations, particularly of nitrogen and sugar, samplings were made as far as practicable at the same period of the day. One sample was collected from each of the sub-plots, as such, nine samples were collected on each sampling date. In sampling, care was taken so that comparable stem leaf internode of different plants was collected. Plants and leaves visibly affected with insects, pests or fungi etc. were avoided as their metabolic process is apprehended to be unusual. Plants (above the ground portion) were chosen at random and cut with a sickle; several cuttings of eight to ten plants were made.

Selection of good plants, for taking the internode was made from these cuttings.

Three stages of growth as prebloom, full bloom and post bloom period of the plant were distinguished and samples were collected in those periods. First cutting was made on June 24, second on June 30 and the third on July 7 (1953). Samples were collected into airtight paper boxes and transported from the field to the laboratory as rapidly as possible with a view to minimize evaporation and enzymatic action. As soon as the samples were brought to the laboratory, they were divided into two parts and each part weighed carefully to the nearest centigram on a torsion balance. The first part was chopped into small pieces by a sharp scalpel and immediately killed in boiling ethyl alcohol for three to four minutes, then removed from the bath at once, and the final concentration of alcohol later brought to 80%, the moisture within the green tissue samples being taken into account. Moisture was calculated on the loss in weight of the green samples when dried at 100-105°C. It has been shown by Laidlaw and Reid (48) that 80% ethanol effects the maximum extraction of soluble sugars. Thus killed the samples were preserved in airtight fruit jars.

The second part was dried in an oven between 75-80°C in a draft of circulating hot air. The dried samples were then ground to pass through a mikro grinder (screen: mikro 039 RD 22G. SS.) and stored in stoppered glass jars for chemical analysis.

At the end of the collection of stem leaf samples, corm samples were also collected in 1953 on July 10, July 25, August 12, August 31 and on September 18. Corm samples were also divided into two parts and prepared in the same way as stem leaf samples for analytical work. In effecting the killing of corms in ethyl alcohol each corm was cut into two parts with a sharp knife and dropped into boiling alcohol.

Composite soil samples were collected from all the nine sub-plots after the collection of stem leaf and corms was completed.

2ND YEAR (1954). In 1954, the second year of this investigation, both Plot I and Plot II were treated with nitrogenous fertiliser in the form of ammonium nitrate in the early growing stage of the plants. As has already been shown on the soil map there were four replications of treated sub-plots with corresponding control sub-plots numbering to 8 (eight), sub-plots of equal areas in contrast to 9 (nine) such plots of 1953.

This year corm samples were collected contemporaneously with leaf stem tissues. Internodes and the corms of the same plant from each individual sub-plot were collected in 1954 on each sampling date. Because stem leaf internodes and corm of the same plant were collected contemporaneously a bunch of plants was dug out by a spade and the selection was made from among them, thus plants of uniform size and height could be chosen. The process of preparing the samples for chemical analysis was similar to that of 1953, except in case of the corms. This year instead of cutting the corms into two parts, they were pressed individually by a pair of pliers and dropped into the boiling alcohol. In this way loss of moisture and other changes were minimised. The experience gained in 1953 indicated the need of refinement of the sampling procedure and the necessity for speed in the killing of the tissue, also the advisability of sampling the third as well as the second internode for a more elaborate study of the processes of translocatinn of the various nutrients.

Contents of nutrients in the growing parts of plant, have been found in the past to be influenced by environment, especially by photoperiod and temperature. Intense light and favourable temperature cause rapid utilisation of nutrients and therefore a low concentration of the unused nutrients would be expected, the opposite conditions tend to cause slow growth and high concentration. However, if growth is checked for a long time the effect may be noticeable even in the low mature portions and the mature tissue may show no need for nutrients. Consequently, tests only on the growing tops may reflect temporary fluctuations in concentration due to changing environment and use of such results might lead to erroneous conclusions. Furthermore, under normal growth if two portions of the conducting tissue of the same plant are studied, an idea of the concentration gradient may be obtained. Therefore, in 1954, instead of collecting only one sample, two samples from the same plant,

(1) the second internode below the inflorescence with the second node and a leaf,

(2) the third internode below the inflorescence with the third node and a leaf,

were collected. Samples were collected at different stages of maturity as in 1953. The first sampling was made on June 25 at the prebloom stage of the plant, the second on July 7 at early bloom, the third on July 14 at full bloom and the fourth on July 22 at the post bloom period. In 1954, the blooming period appeared to extend over a period of about eight days whereas in 1953 it appeared that all the plants came to full bloom in a much shorter period. This discrepancy may be attributed to differences in environmental and biological conditions due to the wide variation in temperature and rainfall in 1954 as compared to 1953. Table II shows a concise weather report during the sampling periods in 1953 and 1954. A more elaborate table of temperature and rainfall has been provided in Appendix B.

Hay samples were also collected on July 22, 1954, from all the eight plots, dried and ground for chemical analysis.

APPLICATION OF NITROGEN. Nitrogen was applied as ammonium nitrate (50 lbs. of $NH_4 NO_3$/acre) on the 15th of May, 1954. Ammonium nitrate was dissolved in distilled water and sprayed uniformly over the plots. There were four replications of this treatment, each one having a corresponding control subplot. The design followed in the collection of samples has been shown diagramatically with the soil map.

TABLE II

VARIATIONS IN THE WEATHER CONDITIONS DURING THE SAMPLING PERIODS IN 1953 AND 1954

Year and month	Date of sampling	Rainfall during 3 days prior to sampling in inches	Total rainfall for the month in inches	Mean temp. for the month in degrees	Hours of sunshine during the month
1953					
May	none	-	3.52	56.26	May 235.8
June	June 24	none	3.54	63.84	
	June 30	0.62	"	"	June 274.9
July	July 7	1.31	3.17	67.37	
	July 10	0.19	"	"	July 258.0
	July 25	0.08	"	"	
August	Aug. 12	0.32	1.92	62.34	
	Aug. 31	-	"	"	August 261.9
September	Sept.18	.30	2.98	55.30	September 172.9
1954					
May	none	-	5.25	52.44	May 187.2
June	June 25	1.10	4.03	63.08	
July	July 6	0.36	1.92	65.87	June 172.1
	July 14	0.08	"	"	
	July 22	0.51	"	"	July 247.0
	July 26	0.37	"	"	
August	Aug..28	0.01	5.63	64.88	Aug. 246.4
Sept.	Sept. 9	0.44	5.76	56.4	
	Sept.10	0.44	"	"	Sept.126.7
Oct.	Oct. 6	0.50	4.03	48.98	
	Oct. 7	0.50	"	"	Oct. 125.6

APPLICATION OF PHOSPHORUS. After harvesting of the hay crop on July 22, 1954, superphosphate was applied on August 10, 1954, to Plot I at the rate of 400 lbs. (20%) superphosphate per acre (80 lbs. of P_2O_5/acre) according to the following key:

Control	N	N	Control
P_o	P_1	P_1	P_1
P_1	P_o	P_o	P_o

P_o = No phosphorus

P_1 = Superphosphate

The fertiliser was mixed with pure sand and spread on the sub-plots by hand. The purpose of this application was to study the effect of phosphate fertilizer on corms and to obtain a measure of the post harvest uptake of phosphorus by the timothy plant.

In each of these sub-plots a small area of two square yards (2x1) was kept, by covering the area with a piece of cardboard a little bigger than two square yards, for experimentation with radioactive phosphorus (P^{32}) and on the rest of the sub-plots marked P_1 phosphorus was applied at the rate described before. Plots marked P_o did not receive any phosphorus but it will be observed that half the number of these P_o P_1 plots had received an application of nitrogen in the previous nitrogen treatment. This enabled the study of the uptake of phosphorus in presence of nitrogen pretreatment. Samples were collected on September 10, and October 7, dried in an oven as before, ground and stored in stoppered glass jars for analysis.

Areas of two square yards each in the sub-plots that had not received superphosphate were treated with solutions of active plus non-active phosphorus in the following manner: 1.6715 gm. of radioactive phosphate ($KH_2 PO_4$) was dissolved in a liter of distilled water, 125 ml. of this stock solution was again made up to a liter and marked (A). 227 gms. of non-active $KH_2 PO_4$ was dissolved in a liter of distilled water and marked (B). 50 cc. of solution A and 125 cc. of solution B were mixed together and made up to 2 liters. Two liters of the solution prepared so was applied to each of the two square yard areas. This treatment corresponds to an application of 400 lbs. (20%) superphosphate/acre (neglecting the insignificant amount of active phosphorus in solution compared to the concentration of $KH_2 PO_4$). The two square yard area was divided into seventy-two equidistant spots by means of a portable wooden frame. The frame was made in such a way that when placed on the ground the inside area covered exactly two square yards (2x1): the wooden frame was fitted with copper cross wires so as to provide seventy-two equal rectangles within the frame. Each of the positions within the frame where the wires cross was supplied with approximately 28 ml. of the prepared solution to ensure uniform distribution of the solution. Samples of corms and new growth of leaf tissues were collected from the sub-plots receiving radio-phosphorus on three different dates - August 28, September 9 and on October 6, 1954, and, after digestion in nitric acid with sulphuric and perchloric acid, immediately counted with a dipping counter.

METHODS

Standard procedures of chemical analysis were adopted in all cases, occasionally with slight modification.

DETERMINATION OF HYDROLYSABLE CARBOHYDRATE.

The reliability of the various Somogyi-Shaffer-Hartman (93, 97) copper reagents for glucose determination in biological materials has long been established. Adaptation of these reagents, replacing iodide-iodate titration by use of an arsenomolybdate colour reagent, to the colorimetric determination of blood sugar has been described by Nelson (67). Woods and Mellon (121) discussed the various interpretations of the reaction. The photometric adaptation of the Somogyi method as evolved by Nelson for the determination of glucose in blood was applied in estimating the total sugar in the hydrolysates of the plant tissue samples collected in this work.

In the determination of total hydrolysable carbohydrate in the oven-dry ground samples 0.2 gm. of the material was hydrolysed with 50 ml. of 1% oxalic acid. Laidlaw and Reid (48) suggested that hydrolysis with 1% oxalic acid gives a quantitative yield of fructose from fructosans. An aliquot of the hydrolysate was then neutralised and clarified as described by Laidlaw and Reid (48). The total sugar was then determined by the Somogyi method (as modified by Nelson). The only change in the use of this method was that double the volume of all the reacting solutions as used by Nelson were taken. Some determinations were, however, made with and without clarifying agent. In most cases it was found that the dilution was sufficient to eliminate the need for clarification especially for the samples collected at comparative early maturity. The purpose of clarification of sugar solutions usually is the removal of non sugar reducing substances. As no difference was found without clarification, this step of clarification was disregarded. All the data on carbohydrate reported in this work were determined without clarification. It was observed that neutralisation was not necessary particularly in case of corm samples where the dilution factor was quite high. An Evelyn Colorimeter with filter 520 mu was used in all cases of sugar determination by this method. This fraction has been designated acid-extractable carbohydrate.

In estimating the alcohol insoluble carbohydrate in the alcohol-preserved samples, the samples were first disintegrated in a waring blender and then extracted with 80% ETOH in a soxhlet for 12 hours. Laidlaw and Reid (48) tried various mixtures of ethanol-water and found 80% ETOH to be the most satisfactory extractant. The author tried different length of extraction time to get all the soluble sugars out of the sample. The alcoholic α-napthol test was positive up to the 10th hour of extraction; to have a fair margin of safety, twelve hours of extraction was adopted in this work. The residue in the thimble was then transferred quantitatively and hydrolysed with 1% oxalic acid and sugar determined as described previously.

CHROMATOGRAPHY

The soxhlet extract was used for the identification of the soluble sugars in the sample by paper partition chromatography. Before the advent of paper chromatography analytical methods for the separation of complex mixtures of sugars depended on the determination of each sugar in presence of one other at least. The predominant sugars in grasses have been known to be sucrose, glucose and fructose (48). In older methods the reducing sugars were estimated in terms of glucose after hydrolysis. Van der Plank (111) has devised a method of titration of fructose after oxidation of aldose with hypoiodite. A complete scheme for the estimation of glucose, fructose and sucrose has been worked out by deMan and de Heus (60). This scheme, however, did not take into account the presence of various oligosaccharides which occur along with the three sugars nor did it allow for small amounts of polysaccharides which may be present in the extract. Laidlaw and Reid (48) developed a more elaborate method for the estimation of the free sugars and fructosan contents. Their method was found to be highly efficient and was adopted in this work.

The alcoholic extract from the soxhlet was evaporated to dryness under reduced pressure and low temperature (40°C) to avoid probable breakdown of sugars. The dried material was taken up with water, clarified, deionised by the passage through resin columns and made up to a definite volume. Then the chromatograms were run and sugars identified. Various solvents and sprays (77) were tried. Benzene-butanol-pyridine-water (1:5:3:3) solvent gave the best separation. The spray used was 1% napthoresorcinol in 2 N Hydrochloric acid. In this work it was found that deionisation was not necessary. Chromatograms run with and without deionisation resulted in the same nature of separation. The R_f values, however, were somewhat lower in case of the samples not deionised. It appears that the concentration of the cations is a limiting factor - the concentration of the cations when low would not interfere in the separation whereas when above a certain limit would require deionisation. The cation concentration (approx. 300 ppm.) in the aqueous solution used in chromatographic separation does not seem to interfere. Better separation was obtained when the filter papers were saturated with moisture over a steam bath after spotting. The saturation can be effected by simply holding the filter paper over a running steam bath for a few minutes. Some of the residues in the thimble were extracted for 10 hours with distilled water after the separation of the alcohol soluble carbohydrates. This extract was dried under reduced pressure and temperature, hydrolysed with 1% oxalic acid and chromatograms were run for the identification of fructosan.

DETERMINATION OF NITROGEN

In the determination of total nitrogen including nitrate-nitrogen in the oven-dry samples, the modified Kjeldahl'macro method as outlined by A.O.A.C. (61) was adopted for digestion. The digest was then made up to definite volume and an aliquot was distilled for ammonia as prescribed for the microkjeldahl method (62).

Alcohol-soluble nitrogen was determined in the alcohol-preserved samples. Samples were extracted with 80% ethyl alcohol in a soxhlet till no more colouring material was left in the thimble. Twelve hours of extraction was sufficient to extract all the colouring materials. Extraction with a fresh quantity of 80% alcohol did not yield any more nitrogen. The extract was transferred to a 750 ml. kjeldahl flask, acidified with 10 ml. of 1:1 H_2SO_4 to avoid loss of nitrogen. 3 gms. of reduced iron powder were then added (86) to reduce the nitrate nitrogen and the extract evaporated to dryness on a water bath. The residue was then digested and ammonia distilled off to determine the total nitrogen.

DETERMINATION OF CATIONS

Conventional methods for the chemical analysis of calcium, magnesium and potassium are very time consuming. Especially in cases where they are present in small quantities, chemical methods are quite unsatisfactory.

Flame photometric methods, involving the use of a Beckman DU spectrophotometer with flame accessory, Model 9200, photomultiplier attachment and an all-metal burner, were chosen to overcome many of the difficulties. The adaptability of this apparatus for analysis on a semimicroscale proved especially useful in analysing samples when only a limited quantity of the material was available. The photomultiplier attachment for the use in the spectral region below 600 mu was of great use in the determination of calcium and magnesium.

The greatest care had to be taken in preparing solutions for reading in the photometer. Perhaps the greatest difficulty that is encountered in a flamephotometric analysis is that of frequent interferences which may influence positively or negatively the intensity of emission of a given element. Almost any substance can modify the emission of an element when present in high concentration. Generally the difficulties may be overcome in one of several ways, such as by correction for the concentration of the interfering substances, by addition of the interfering substances to the standards, or by removal of the interfering substances. A number of workers have reported interference by different materials (2, 10, 16, 18), and although only a few reports have been available using the specific equipment (23, 28, 87) worked with here, these have served as very useful guides in designing suitable methods for the determinations of the cations with the flamephotometer.

A wet ashing technique with conc. HNO_3 and HCl acids was employed for the digestion of the sample (0.25 gm. in case of the 1953 samples and 0.30 gm. in case of 1954 samples). The digestion procedure was employed to convert all the salt finally to chlorides because the standard solutions were used as chlorides. The chloride residues were dried on the steam bath and taken up in 25 ml. 0.2 N HCl by heating for a minute and a half on a hot plate. After cooling 10 ml. aliquots were passed through columns (4 mm in diameter by 8 cm. in length) of Dowex-50 cation exchange resins saturated with NH_4^+ ions. The solutions were percolated through at the rate of two drops a minute. Then the columns were washed thoroughly with distilled water and the cations eluted with 60 ml. of 5N NH_4NO_3 solution passed through at the rate of one ml. per minute. Completion of absorption by the columns and elution by NH_4NO_3 was occasionally verified by reading a sample from the last few ml. of the elute in the flame photometer and comparing the reading with that of the eluting solution. The eluted cations were converted to chlorides in the same manner as for the plant materials. The exchange resin method of purification was developed by DeLong and MacKay (17) after the serious interference of phosphorus on the emission of calcium and magnesium was observed. DeLong and MacKay suggest that the estimation of potassium should be made from the original solution because its ready ionization from the resin did not permit complete absorption on the small column used. The author, however, found that the original tissue digest and the elute gave the same reading for potassium on the photometer.

It was found necessary to examine the stability of emission with known concentrations at intervals and to redraw the standard curves as often as the unknown solutions were read so that an average condition under which the apparatus was used could be attained.

In the preparation of the standard, a stock solution was prepared in 0.2 N HCl from salts of high purity with the three ions in solution (potassium, calcium and magnesium) containing a little more than the expected proportion in the samples and thus from the stock a series of dilutions was made to construct the lower points in the curve.

In operating the flame photometer for the determination of a given element the sensitivity control was set at one turn less than the counter clockwise extreme and the slit width was then adjusted with the strongest standard solution at proper wave length to give maximum emission of 100. After attaining a suitable slit width further adjustment to make this standard read exactly 100, was effected by slight turning of the sensitivity control. Following this adjustment the other solutions of the series were read and the standard curves were drawn by plotting emission against concentration. Before running a series of known or unknown solutions, instrumental stability was checked with the strongest solution and at least two closely agreeing readings were secured. The photomultiplier attachment was essential in the determination of calcium and magnesium.

Data concerning the concentrations of the standard solutions, along with the approximate instrumental setting used with the metal burner atomizer and the photomultiplier attachment, are given in Table III. This setting will, however, vary with the individual worker.

TABLE III

CONCENTRATION RANGES OF THE STANDARD SOLUTIONS

AND THE

INSTRUMENTAL SETTING USED IN THE DETERMINATION

OF CATIONS IN THE TISSUE SAMPLES

Element	Conc. of the strongest solution	Wave Length	Sensitivity turns from counterclockwise extreme	Slit width	Selector switch position	Phototube positioning Knob (at)	Photo-cell
K	400 ppm	767	1.0	0.025	0.1	3	Red
Ca	160 "	422.1	1.5	0.0125	1.0	2	Blue
Mg	160 "	285.1	2.0	0.03	0.1	2	Blue

Note: The oxygen and acetylene pressures were at 15 and 3.5 p.s.i. respectively.

DETERMINATION OF PHOSPHORUS

Total and acetic acid-soluble phosphorus were determined on all the oven-dry samples. All phosphorus determinations were made colorimetrically with colour development by the Truog-Meyer procedure (108). In the case of the plant materials easily-soluble phosphorus was extracted with 2 per cent acetic acid followed by oxidation of the extract with hydrogen peroxide. In the extraction 0.2 gm. samples were shaken for five minutes (by mechanical shaker). The total phosphorus content of plant samples were determined following oxidation with nitric and perchloric acid. Easily-soluble phosphorus was extracted from the soil samples by the method of Bray and Kurtz (9) for adsorbed phosphorus. The method of extracting soluble phosphorus from green plant materials to correlate the content of nutrient with the phosphorus supplying ability of the soil was first enunciated by Emmert (20). He used charcoal for attaining a clear solution, in this work hydrogen peroxide was used instead. An aliquot of the extract was treated with about 6-10 drops phosphate-free hydrogen peroxide for the oxidation of the organic matter and evaporated to dryness on a steam bath. Care must be taken to drive off the last trace of hydrogen peroxide. The residue was taken up with water and the colour was developed at a definite volume.

RADIOACTIVE PHOSPHORUS (P^{32})

As it was decided to apply active orthophosphate phosphorus to the field in solution, chemical assay of the forms of phosphorus in the neutron bombarded KH_2PO_4 received from Chalk River Laboratories became important to investigate if the phosphate was wholly in the desired chemical form. Non-active KH_2PO_4 is soluble in cold water (solubility - 33 gms./100 ml. water at 25°C) whereas K-pyrophosphate and K-metaphosphate are very slightly soluble (pyro - slightly and meta - insoluble) in water. It was supposed that the two grams (the total weight received) of the irradiated orthophosphate (as indicated on the shipping document of the material) would readily go into solution. However, experience showed that it did not. It was clear that the orthophosphate had been converted into insoluble form during irradiation. The brown colour of the irradiated sample suggested contamination or conversion of some of the phosphorus to the elemental state. It has been reported by Fried and MacKenzie (24) that $KH_2P^{32}O_4$ obtained by neutron pile bombardment of KH_2PO_4 is soluble in water, but since the sample of $KH_2P^{32}O_4$ received from the Chalk River Laboratories did not go into solution even in acid - hot or cold, dilute or concentrated - it was concluded that the orthophosphate must have undergone a major change during the neutron bombardment. Dilute and concentrated nitric acid seemed to have no or very little effect. Next, the solution containing the irradiated solid was reduced to a very small volume and treated with liquid bromine and heated under an ordinary heater lamp. The heater lamp was so adjusted that the process of volatilisation was slow. Still one treatment with bromine was not enough. Repeated treatment with bromine appeared to have a significant effect: apparently more than half of the material went into solution. Treatment was continued for 96 hours and the supernatant liquid was decanted into a volumetric flask. The residue was washed several times and the washings were taken in with the original liquid in the flask and made up to volume. Total phosphorus was determined in an aliquot of this solution and the total phosphorus taken into solution was calculated. This was equivalent to 1.6715 gm. of KH_2PO_4. After that the non-orthophosphate phosphorus content of the solution was assessed by the method outlined by MacKenzie and Borland (58). It was found that all the phosphorus in solution was in the orthophosphate form. Solutions were then prepared for application in the field as described earlier.

Corm and plant samples from P^{32}-treated plots were collected and were digested first with nitric acid in an Erlenmeyer flask with cover. A preliminary test was made for probable escape of phosphorus, when treated with conc. HNO_3 during digestion. Filter papers were cut in a circular shape and placed on the neck of the Erlenmeyer flask containing radioactive solutions and materials with conc. HNO_3 and the flask heated to 70°C.

Vigorous reaction started and brown fumes of NO_2 came out. All the fumes were arrested and passed through the filter paper. When the reaction subsided, the piece of filter paper was tested for activity in a Geiger counter. No activity could, however, be recorded. This showed that no significant loss of phosphorus occurred when the samples were digested with HNO_3 in this way.

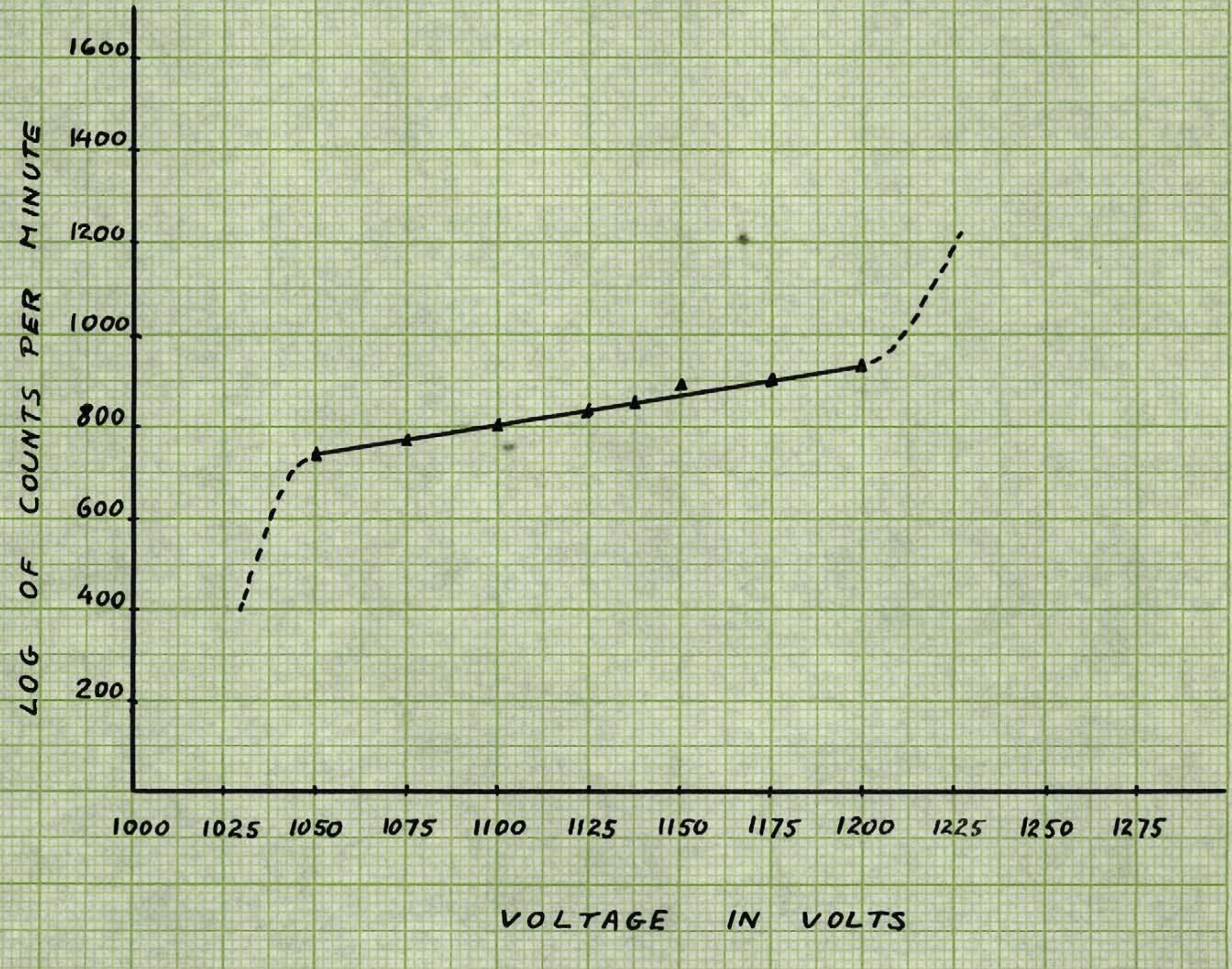
Before digestion with HNO_3, green samples were divided into two parts, one part was dried in an oven between 100-105°C for the determination of moisture and the other part taken for digestion. Subsequent to HNO_3 treatment equal volumes of 1:1 sulphuric acid and HNO_3 and a few drops of perchloric acid were added for complete digestion of the sample which was colourless. Care must be taken so that in all cases a definite amount of acid is added to have a uniform concentration of acid solution all through the determinations because differences in concentration may lead to interferences in counts with a dipping counter, thus giving erroneous counts. After digestion the solution was transferred into a volumetric flask and made up to volume; 9 cc. of the solution was then counted with a dipping counter.

The counter used was a dipping counter B6 type. Phosphorus (P^{32}) has a beta radiation with a maximum energy of 1.7 meV and for this radiation the tube has an efficiency of 8 to 10%. The precautions for working the counter enunciated by Veall (112) were followed. The B6 (immersion type) dipping counter is a thin-walled counter tube of borosilicate glass. Beta counts may be taken by immersing the tube directly in a sample liquid. As geometry is very important in scaling the counts, the same geometry was kept for all the measurements. The rack and pinion device in the counter used was an advantage in keeping the geometry for all practical purposes the same in all cases. The recommendations by Solomon and Ester (96) were followed regarding geometry and counter efficiency.

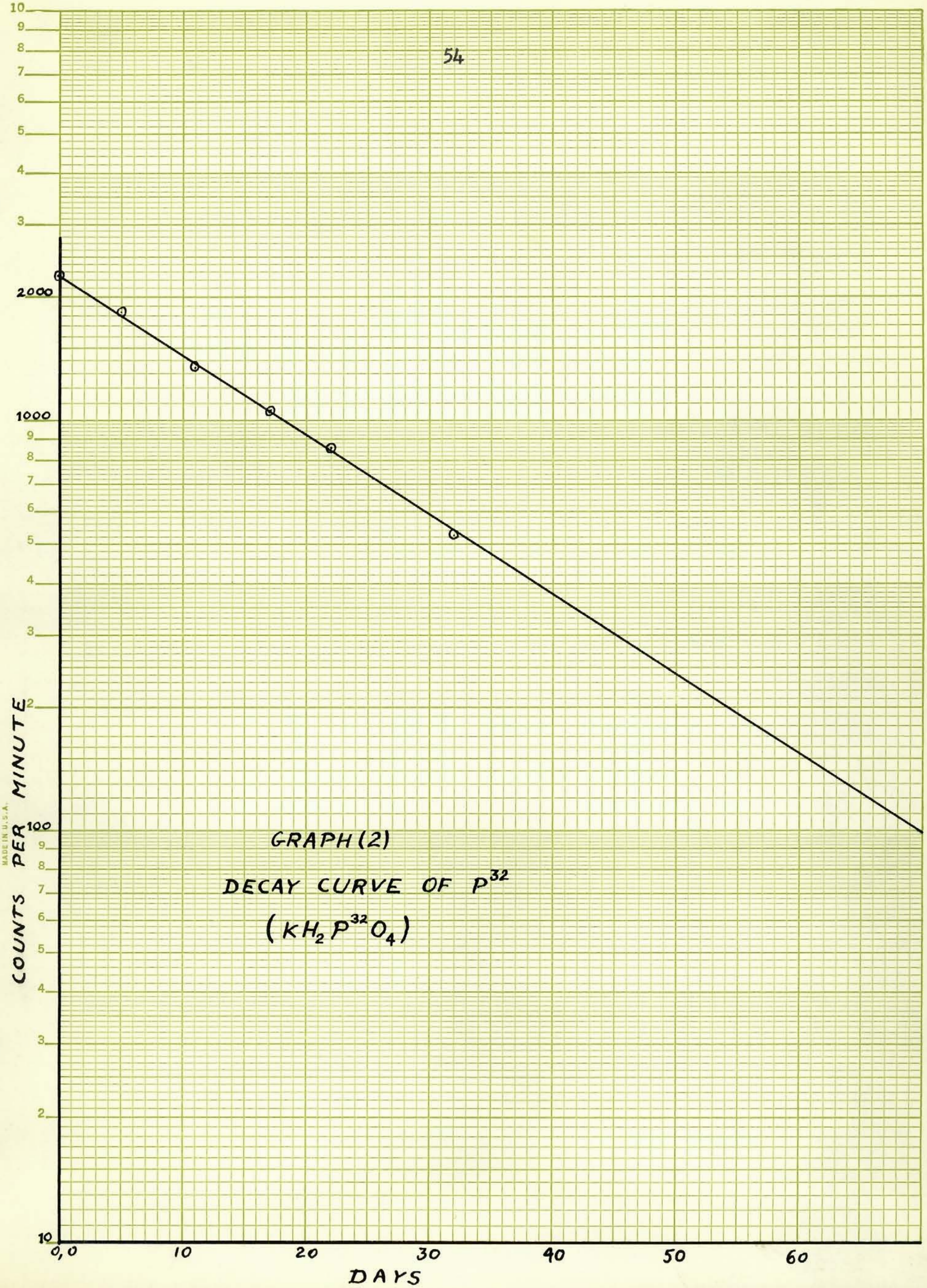
The operating potential for a beta counter of the type used is generally around 1050 volts. However, in this case the characteristic curve for the counter was drawn and the mid-point of the plateau was taken as the operating potential (See Graph I), which was found to be around 1140 volts.

GRAPH (1)

THE CHARACTERISTIC CURVE FOR DETERMINING THE PLATEAU OF THE COUNTER USED [B_6]



The radioactive assays for P^{32} extended over a period of about three months and P^{32} has a half life of 14.7 days. It was therefore necessary to have a system established for interpreting the activity on a common basis. This was effected by means of the decay curve of P^{32} (See Graph 2) drawn as a straight line with the log of the C/M plotted against days. All the readings (after the deduction of the background count) were converted to "O" day from the reading taken from the decay curve.


GRAPH (2)
DECAY CURVE OF P32
(KH2P32O4)
COUNTS PER MINUTE
DAYS
2000
1000
100
10
0,0
10
20
30
40
50
60
KEUFFEL & ESSER CO.
358-71
Semi-Logarithmic, 3 Cycles X 10 to the inch, 5th lines accented.
MADE IN U.S.A.

EXPERIMENTAL RESULTS AND DISCUSSION

SECTION I: COMPOSITION OF UNFERTILISED PLANT

Table IV presents the mean moisture content of the different tissue samples at three periods of maturity with the corresponding rainfall data during three-day periods prior to the dates of sampling. This table shows that there is a general tendency of the moisture content in the stem leaf samples to be progressively lower as the plant matures; the early growing stage has the highest moisture content in both the years. In 1953 the moisture content in the post bloom is not different from the blooming period; this can be attributed to the fact that the post bloom period in 1953 had a much higher rainfall - more than double - than the blooming period. That the moisture content is at least partially dependent on the rainfall may be found from a comparison of the data obtained for the same period of growth during 1953 and 1954. However, rainfall may not be the only factor in controlling the moisture content of the plant. Temperature, hours of sunlight and other environmental factors may play important roles in this connection. From a consideration of the data obtained on the moisture content of the stem leaf samples both in 1953 and 1954, it seems definite that the moisture content of the stem leaf of the timothy plant decreases as the plants mature; this is further corroborated by the fact that the third internode (the more mature part) has always a lesser moisture content than the second internode (comparatively less mature part). This contention appears to hold good in spite of the irregular variations in the rainfall at different stages of growth.

TABLE IV

AVERAGE MOISTURE CONTENT (AS PER CENT OF GREEN TISSUE) OF TIMOTHY STEM LEAF AND CORM SAMPLES AT DIFFERENT PERIODS OF GROWTH

1953 samples			1954 samples		
Stages & dates of sampling	Mean moisture content	Rainfall# in inches	Stages & Dates of sampling	Mean moisture content	Rainfall# in inches
2nd internode			2nd internode		
(1) Prebloom 24.6.53	66.08	None	(1) Prebloom 25.6.54	71.93	1.10
(2) Early bloom 30.6.53	64.00	0.62	(2) Early bloom 6.7.54	67.60	0.38
(3) Post bloom 7.7.53	64.00	1.31	(3) Full bloom 14.7.54	64.56	0.08
			(4) Post bloom 22.7.54	58.43	0.51
			3rd internode		
			(1) Prebloom 25.6.54	71.76	1.10
			(2) Early bloom 6.7.54	67.50	0.38
			(3) Full bloom 14.7.54	64.34	0.08
			(4) Post bloom 22.7.54	55.95	0.51
Corms			Corms		
(1) July 10'53	61.43	0.53	(1) July 14'54	65.36	0.08
(2) July 25'53	64.80	0.37	(2) July 22'54	60.00	0.51
(3) Sept.18'53	59.86	0.26	(3) Sept.10'54	62.79	0.44
			(4) Oct. 7'54	62.25	0.50

Total rainfall during three days prior to sampling

Moisture content in the corms seems to have a completely different picture than that in the stem leaf. The variations are very irregular and cannot be assigned to one single reason at any one stage. Again, there was sampling difference between 1953 and 1954 corms. The trend of the variation in moisture content in the corms cannot be ascertained without further experimentation.

Table V presents the variation in the dry weight of the second and third internodes and corms at different stages of maturity collected in 1954. It will be observed that with the progessive maturity of the corms the dry weight of individual corm decreases whereas the dry weights of the individual internodes increase with growth. As comrs were collected contemporaneously with the second and third internodes of the same plant, the total number of corms collected in one sample gave a measure of the total number of internodes collected in that sample. This figure was taken into account in calculating the dry weight of the individual internode.

TABLE V

AVERAGE WEIGHT (OVEN DRY) IN GRAMS OF THE TIMOTHY SECOND AND THIRD INTERNODES & CORMS AT DIFFERENT PERIODS OF GROWTH

(a)

2ND INTERNODE

Date	25.6.54	6.7.54	14.7.54	22.7.54	D.R.S. between dates at P_{05}	P_{01}
No Nitrogen	0.140	0.150	0.155	0.169		
Coefficient of variability	6.81	9.23	7.52	10.81	0.0047	0.0091
Nitrogen applied	0.146	0.155	0.162	0.180		
Coefficient of variability	7.11	7.32	6.51	7.43		

D.R.S. for treatment at P_{05} = 0.0038
P_{01} = 0.0073

(b)

3RD INTERNODE

Date	25.6.54	6.7.54	14.7.54	22.7.54	D.R.S. between dates at P_{05}	P_{01}
No Nitrogen	0.148	0.156	0.160	0.172		
Coefficient of variability	7.11	8.28	8.11	8.34	0.0053	0.0102
Nitrogen applied	0.150	0.159	0.165	0.181		
Coefficient of variability	5.63	7.11	5.13	4.83		

D.R.S. for treatment at P_{05} = 0.0037
P_{01} = 0.0072

(c)

CORMS

Date	Mean values for a single dry corm	Coefficient of variability	Differences in successive pairs
June 25 1954	0.4162	4.36	
July 6 1954	0.3828	1.15	0.0334
July 14 1954	0.2951	2.35	0.0877
July 22 1954	0.2409	2.11	0.0542

D.R.S. for P = 0.05 is 0.0423

NITROGEN AND ACID EXTRACTABLE CARBOHYDRATE

The direction and significance of the changes in dry weight in percentages of total nitrogen and acid extracted carbohydrate (simple sugar plus 1% $H_2C_2O_4$ hydrolysable carbohydrate) occurring in the tissues sampled during the blooming period are shown in Table VI. Dry weight changes were not determined in 1953. In Table VI the variations are shown in terms of percentage changes between dates of sampling. The data upon which the values given in this table (VI) are based are given in Table I and II of Appendix C.

TABLE VI

CLIMATIC DATA AND PERCENTAGE CHANGES IN COMPOSITION

Interval	Rainfall in inches	Sunshine hours	Tissue	Percentage change D.W.	Total N	acid extracted carbohydrate
May 1-June 17(53)	5.92	434	Second			
June 17-24 (53)	0.00	76				
June 24-30 (53)	0.62		Inter-			
Prebloom to bloom				?	+29.1**	-25*
June 30-July 7(53)	1.67	62	node			
Bloom to post bloom				?	-22.6**	-7*
Total May 1-July 7 (53)	8.21	572				
May 1-June 18(54)	7.93	319	Second inter-node	+7*	-24.7**	+28.0*
June 18-25 (54)	0.00	52				
June 25-July 6(54)	1.80		Third inter-node			
Prebloom to early bloom				+5	-23.1**	+45.5*
Total May 1-July 6 (54)	9.73	421	Corm	-8	-17*	+5.2
July 7-14 (54)			Second inter-node	+3	-17.3*	-21.6*
Early bloom to full bloom	0.24	81	Third inter-node	+2.6	-9.8*	-10.4*
			Corm	-23*	+8.5*	+4.4
July 15-22 (54)	0.52	59	Second inter-node	+9*	-11.1*	+2.5
Full bloom to post bloom			Third inter-node	+7.5	-33.5**	-5.7
			Corm	-18*	-0.59	-24.4*

N.B.*significant at P=.05; **significant at P=.01

Table VI shows that the stem leaf tissues continuously increased in dry weight during the 1954 sampling period whereas the corms continuously decreased in dry weight particularly during the last two intervals.

It also is shown that the nitrogen content of the tissue of the second internode behaved quite differently in the two seasons, the prebloom to bloom increase in 1953 not being found in 1954. The decreases in nitrogen content of both the second and the third internode stem leaf tissues in 1954 being uniformly greater than the increases in dry weight it is concluded that nitrogen was being translocated from those tissues during the 1954 blooming period. It seems probable that a part of the translocation was in the upward direction to the developing inflorescence. However, during the interval July 7th to July 22nd, 1954, the corm tissues increased in total nitrogen content to a considerably greater extent than they decreased in dry weight. Evidently the tissues accumulated nitrogen during that period. It is not known to what extent their increased content of this element was due to downward translocation from the stem leaf and to what extent it was due to uptake of nitrogen from the soil.

Like the data for total nitrogen, the values shown in Table VI for the changes in acid-extracted carbohydrate content of the tissues of the second internode during the prebloom to bloom intervals are in an opposite direction in 1953 from that in 1954. These seasonal differences in nitrogen and carbohydrate contents may be related to the considerable climatic differences during the early growth period (May 1st to the last week in June) of the years 1953 and 1954. Table VI shows that this part of the 1953 growing season was definitely drier and more sunny than the corresponding period in 1954. The more complete data on carbohydrate changes obtained in the latter year show that both the second and third internode samples accumulated acid extractable carbohydrate during the prebloom to early bloom interval and lost it during the early bloom to full bloom period. It appears probable from the data of Table VI that the corm tissues decreased in content of acid extractable carbohydrate in the full bloom to post bloom interval in 1954 and that in the preceding interval, the increase in the amount of this component in the corms may have been due to downward translocation, the loss for the third internode at this time being larger.

Differences in the protein content may be attributed to the differences in the physiological condition of the plant. In the normal growing plant, provided the available nitrogen is not limiting, the protein content of a given leaf is maintained at a relatively constant level over a long period of time. This has been shown for example by the nearly equal protein concentrations found in successive leaves of barley (30) and tobacco (33) and many other species. Walkley (116) has followed the diminution of protein in leaves of nitrogen-deficient barley plants, the fourth leaf being analysed periodically from the time of maturity onward. Over a period of twenty days protein content of this leaf dropped from 1.82% to 0.19%. Similar results have been obtained with cotton (81).

Diurnal fluctuations in the protein level have been observed by several workers. These fluctuations are small. Exposure of leaves on the intact plant to periods of prolonged darkness induces more extensive protein loss than is found during a single night (120). Young leaves increase in protein continuously while old ones lose protein in a similar condition. It appears from the data of Table VI that similar losses take place in the timothy internodes due to translocation.

A further factor regulating the leaf protein level is the water stress to which the plant is subjected. Mothes (65) has reported that high water stress causes protein hydrolysis in leaves and this view is concurred in by Wood and Petrie (122). The effect is observed only when the leaf is attached to the plant and may have to do with the transport of soluble nitrogen products of protein hydrolysis away from the leaf, i.e. the effect may be primarily on translocation rather than protein hydrolysis.

Increased rainfall in 1954 seems to have caused high nitrogen content in the prebloom stage. Samples taken on other dates have similar moisture content as reported by other workers (54). The high nitrogen content in the early bloom stage of 1953 over that in 1954 cannot be explained only from the water stress. Perhaps the rate of translocation was low during that period as effected by the rainfall. It appears that factors other than water stress per se are operative in the regulation of the stem-leaf nitrogen content.

Examination of the literature shows that though various workers have conducted experiments on evaluating the nutrition of the timothy plant, corms have never been studied in detail. Harper and Phillips (34) asserted that the haplo-corms were the storage organs by showing their striking differences in composition with the adjacent lower stalk, but with regard to nitrogen they said that the storage is comparatively small. In this work though the corms contained less percentage nitrogen than the stem-leaf there was definite accumulation of nitrogen in the corms as shown in Table VI. However, this will not give an absolute measure in proving the adequacy of the corm nitrogen to contribute to the nitrogen content of the stem leaf because the nitrogen content of the corms may be continuously replenished from roots and soil during the growing season. The contention is corroborated by the fact that the decrease of nitrogen in the second internode in successive days of growth is much more than the corresponding decreases in the corms.

Results with regard to acid-extractable carbohydrate obtained in this work in 1953 are similar to those obtained by Phillips and Smith (80). They found a more pronounced decrease in the total hydrolysable sugars of the timothy plant with maturity; their method of hydrolysis, was, however, different. They differentiated 0.2N H_2 SO_4 and H H_2 SO_4 hydrosylable carbohydrate. The 0.2N H_2 SO_4 hydrolusable carbohydrate consisted of glucose, fructose, sucrose and fructosan and was somewhat comparable to author's acid extractable carbohydrate.

A comparison of 1953 and 1954 data on acid-extractable carbohydrate suggests that climatic variations may have a profound effect on the composition of the timothy plant with regard to this fraction, at least when grown under the conditions described in the section on experimental methods and materials. From the weather report during 1954, it will be observed that in this year the rainfall was appreciably more than the previous year and extended over longer period than usual, and that the light intensity during the daylight period was also low. Thus it is evident that the photosynthetic process in 1954, especially during the sampling period, was affected as shown by a lower figure for acid extractable carbohydrate in 1954 than 1953.

Waite and Boyd (114), working at the Hannah Dairy Research Institute, suggest that before considering the carbohydrate values in any set of determinations in herbage plants like timothy, it is necessary to note the difference in the weather conditions between years and their effect on the growth of the plant. They found that the spring of 1952 was warmer and drier than that of 1951, and that this was more effective in promoting growth than the higher rainfall in 1951.

Homb (40) also reported results of this nature. Variations appreciable from year to year were found by Homb in the sugar percentage of herbage, such variations being often considerable within the year. However, no definite correlations with the stage of maturity was established by him, since both positive and negative correlations were found for the various years. He found that the mean content of all samples from the first cutting was 15.2% sugar in grasses (one being timothy). He remarked that it was difficult to find a valid explanation of the variability in the sugar contents of the plant, which was considerable in his investigation. He found that an interaction existed between the content of crude protein and sugar in such a manner that circumstances conducive to liberal uptake of nitrates tended to produce crops lower in sugar. In this work no such negative correlation between the nitrogen content and acid extractable carbohydrate could be established.

Svanberg and Ekelund (100) have pointed out that under conditions of liberal nitrogen supply large quantities of sugar are required for the formation of amino acids. If at the same time photosynthetic processes are also low, very little sugar reserve will be built up in the plant.

Very few investigations have been reported with regard to acid-extractable carbohydrate of the timothy corms. Harper and Phillips (34) analysed timothy bulbs but their methods of hydrolysis and differentiations of fractions were very different from that of the author. The figures obtained by them are not comparable with those of the author especially because the stage of maturity of their corms are not known.

SIMPLE SUGARS

The carbohydrate extracted by refluxing with one per cent oxalic acid may be assumed to include saccharides of low molecular weight (hereinafter called simple sugars) plus the more easily hydrolysable polysaccharides of the tissue. Determination of one per cent oxalic acid-hydrolysable carbohydrate also was made after exhaustive extraction of the stem leaf tissues with 80 per cent aqueous ethanol for removal of simple sugars. The data obtained on the alcohol insoluble acid hydrolysable carbohydrate are given in Table III of Appendix C. The differences between the values obtained by the two determinations, the one without and the other with preliminary alcoholic extraction provide an estimate of the amount of simple sugars present. The amounts of simple sugars present (as per cent of dry weight) and the percentage changes in the amounts of simple sugars, alcohol insoluble acid extractable carbohydrate and total acid extractable carbohydrate in the tissue of the

the second and third internode samples collected in 1954 are presented in Table VII. Along with the data on carbohydrate changes the accompanying changes in dry weight also are recorded to facilitate comparison.

TABLE VII

CHANGES IN CONTENT OF CARBOHYDRATE FRACTION IN 1954

Interval	Simple sugars % in D.M.	Simple sugars & change	Alcohol insoluble acid extractable % change	Total acid extractable % change	Dry matter % change
Second internode					
June 25 to July 6	2.77 2.53	+11*	+34*	+28*	+7
July 7 to July 14	2.53 1.34	-47*	-14*	-22*	+3
July 14 to July 22	1.34 0.00	-100*	+21*	+3.3	+9
Third internode					
June 25 to July 6	2.58 3.99	+55*	+46*	+45*	+5
July 7 to July 14	3.99 0.62	-86*	+24*	-10*	+2.6
July 14 to July 22	0.62 0.11	-84*	nil	-5.4	+7.5

*Significant at P=05

Inspection of Table VII shows that with the exception of the change in simple sugars of the second internode during the first interval, the fluctuations in simple sugar content are greater than those for the more complex carbohydrate fractions. This suggests that the information provided by the extraction of this fraction well may justify the extra labour required, especially as is shown later, the estimation of alcohol soluble nitrogen appears to be valuable in the assessment of nitrogen status. Further investigation of the alcohol-soluble fraction seems warranted.

It is seen that with the progress of maturity of the plant, the simple sugar content of the plant is decreasing except in the early growing stage. To verify this conclusion chormatograms were run on the alcohol extract of the stem-leaf samples after clarification and evaporation under reduced pressure. Three fractions - glucose, fructose and sucrose were identified. No quantitative determination was made on the samples but colour densities were measured in a densitometer as a measure of relative concentration. It was found that, in the early stages of growth, the sucrose spot had the highest density, and that as the plant matured this spot became less dense indicating the loss of sucrose. After the removal of the soluble sugars with 80% ethanol in the soxhlet, the alcohol insoluble residue is expected to contain the fructosan fraction of the hydrolysable carbohydrates of the plant because fructosan of the timothy plant has been reported to be insoluble in 80% ETOH. That this alcohol insoluble fraction could be considered as fructosan was proved by chromatography. After the removal of the alcohol-soluble fraction of the carbohydrate, the residue was extracted with water, evaporated to dryness, hydrolysed and neutralised and then chromatograms were run. Only one spot, which had the same Rf value as fructose, could be identified. The intensity of this spot decreased with the maturity of the plant.

The variations in the inorganic constituents of the samples collected in 1953 and 1954 have been presented in Table VIII. Dry weight changes also have been included in this table for comparison. The data upon which these values in Table VIII are based, are given in Tables IV, V, VI, VII and VIII of the Appendix C.

TABLE VIII

PERCENTAGE CHANGES IN ORGANIC NUTRIENT CONTENT AND IN DRY WEIGHT

Interval	Tissue	HAc-sol. P	Total P	Total Mg	Total K	Total Ca	D.W.
June 24-30 '53 Prebloom to early bloom	Second internode	Nil	-5.9	+3.5	+2.5	-4.5	?
June 30-July 7 '53, Early to post bloom	Second internode	+17*	+1	+11	-5.4	+5.6	?
June 25-July 6 '54, Prebloom to early bloom	Second internode	+6	+5	+55*	-2.7	-6	+7
"	third internode	+16*	+21*	+56**	-6.6	-28*	+5
"	Corm	+34.2*	+10.9*	+3.2	-5	+31*	-8
July 7-14, '54 Early to full bloom	Second internode	-18*	+6.8	-83**	-38**	+36**	+3
"	third internode	+3	-6	-87*	-39**	+45**	+2.6
"	Corm	-4.2	+25.3*	+86.4*	-5	+32*	-23
July 14-22, '54 Full to post bloom	Second internode	-2.4	+1.6	nil	-13*	+12*	+9*
"	third internode	-5.4	-3.6	-26	-13*	+10*	+2.5
"	Corm	-2.4	-2	+37.5*	nil	+11	-15*

*Significant at P=05 **significant at P=01

It will be observed from Table VIII that the nature of variations of HAc-soluble phosphorus content of the second internode in the two years is different. In 1953 the soluble phosphorus content of the second internode is essentially constant during the prebloom and the blooming period, then rises to a relatively higher figure in the post bloom period. This suggests that during the prebloom and blooming period, the soluble phosphorus is utilised or translocated as fast as it is being taken up from the soil, whereas during the post bloom period, there is an accumulation of phosphorus in the second internode. In 1954, after a fairly large increase during the early bloom to full bloom period, the soluble phosphorus decreases. It has already been mentioned in the previous discussion that the year 1954 was characterised by higher rainfall, lower temperature and shorter sunshine periods over 1953. The lower soluble phosphorus content in the more mature stages may thus be due to the weather conditions.

It may also be relevant to point out in this connection that there were two blossoming periods in 1954 and that a rapid mobilization of phosphorus to the reproductive organs may be expected during blossoming and seed setting. Further, as the later bloom (full bloom) was greater than the former (early bloom) depletion of phosphorus in the second internode may well have been delayed in 1954. Again, the flower heads were found to be larger in 1954 and more seed was expected to be formed, thus greater depletion of soluble phosphorus content of the second internode in 1954 than in 1953 would be expected.

Examination of the data on total phosphorus (Table VIII) content of the stem leaf samples shows once more an apparent effect of season oh the composition of the timothy plant. Visual observation indicated plants larger in 1954 than in 1953 and second internode consistently lesser in total phosphorus in 1954 than in 1953.

The analytical data obtained in this work for the total phosphorus content in the stem leaf samples (Table V, Appendix C) is much lower than those obtained by other workers. From the summary compiled by Beeson (4), it will be seen that the total phosphorus content of the whole plant in the full bloom stage ranges from 0.17 to 0.31 per cent and in the post bloom stage from 0.17 to 0.41 per cent and the total phosphorus of hay after harvesting was found to be 0.127 per cent. From this point of view it appears that the soil on which the timothy plants were grown in this work needed application of phosphorus fertilizer.

It will be observed that in both the years there is a general tendency of percentage increase of phosphorus in the corms. 1954 data proves this contention concluwively.

The total phosphorus content (Table V, Appendix C) obtained for the corms in both the years is much lower than the values obtained by other workers. Trowbridge et al (107) obtained much higher figures for the total phosphorus content of the timothy bulb. The values obtained by them for the timothy bulbs ranged from 0.67% to .44% between May 23 and July 20 (1908). Their analytical data showed progressively lower value for the content of total phosphorus as the corms mature but the lowest value obtained in their experiment was higher than the highest value obtained in this work. This further suggests need for fertilization with phosphorus.

Inspection of Table VIII once again indicates a significant seasonal difference in the total magnesium contents of the stem leaf internodes and corm samples. It appears that the magnesium content (Table VI, Appendix C) in the stem leaf samples in 1953 varied sparingly whereas in the 1954 samples there was a great variation at different stages of growth of the plant, especially striking was the apparent accumulation of magnesium in the stem leaf tissue at the early bloom stage in 1954 with subsequent rapid depletion. The data indicates a rapid upward translocation of the element during the flowering and seed setting stage. Price et al (85) have reported relative constancy of magnesium content of timothy with variation of growth site but such a large seasonal variation as that found by the author for the 1954 crop does not appear to have been recorded formerly. Robinson et al (90) found that the magnesium content of timothy hay cut at the full bloom stage varied from 0.09% to 0.169% but on the other hand the range in the magnesium content of the tissue sampled by the author in 1954 has almost as large an amplitude as that reported by Beeson (4) for timothy hay grown under widely variable conditions. The 1954 data suggest that a sufficient supply of the element magnesium during the reproductive phase of the timothy plant may be highly important especially during flowering and seed setting. Whatever the significance of this mobilization of magnesium, comparison of the data obtained for the soluble phosphorus content of the tissues with those for magnesium fails to reveal the often-postulated relationship of the latter to the mobility of phosphorus. It has been reported (37) that low nitrogen status is often accompanied by low magnesium status, that magnesium deficiencies occur in crops on soils low in nitrogen, and that the effect of magnesium deficiency may be removed for such crops by the application of nitrogenous fertilizer (43). Hence it may be significant that the timothy plants of 1953 crops showed both higher nitrogen and higher magnesium status than those of the 1954 crop.

Comparison of the 1953 and 1954 data in reference to the climatic conditions suggests that climatic conditions affect the mobilization and transport of this element.

Examination of Table VIII with regard to the potassium content indicates a decline in potassium of the third internode. There is a significant loss of potassium from the second internode during early to post bloom period. The values obtained for the potassium content (Table VII of Appendix C) seem to be in the optimum range as reported by other workers (3, 15, 42, 50). The corms and the pre-bloom periods of the second internode do not suffer any loss of potassium content.

The changes in total potassium and total nitrogen contents of the stem leaf tissues collected in 1954 are in the same direction. Statistical analysis, however, shows that the correlation is not significant.

Inspection of Table VIII reveals a percentage increase of calcium content of all the tissues analysed except stem-leaf third internode during prebloom to early bloom in 1954. There is a decline of calcium in the early bloom period in both the years, but it has been found in 1954 that this loss of calcium is not real and may be partially due to dilution. It appears that during the early reproductive period the vegetative growth of the plant is fast and the rate of calcium uptake is not as fast as the rate of vegetative growth. The decline at early bloom is followed by an increase during the latter part of the reproductive phase. This behaviour differs from that found by Plummer (84) who, however, analysed the whole plant. Data compiled by Beeson (4) also are of interest in this connection.

Thus Beeson showed from the average of a large number of analyses, that the calcium content of the timothy hay harvested when beginning to head was about 0.23 per cent. Variations in the content of this element at the blooming stage were attributed by Beeson to the influence of the soil on which the crop was grown. The data obtained in present investigation indicate similar variability due to climatic variation.

The results obtained in the investigation of the seasonal variations in composition of the timothy plant, more especially the marked differences in the data obtained in 1953 and 1954, make it clear that sampling of this crop for fertility diagnosis is more difficult than had been anticipated. It is evident that further investigation of these variations is desirable. However, a part of the difficulty may be a result of the selection as the sampling period of the interval inclusive of the reproductive phase. It is suggested that, in further study of the problem of sampling for diagnostic purposes, the sampling interval be extended to include both earlier and later periods in the growth cycle. On the other hand, the sampling interval chosen and the tissues selected for analysis in 1954, favoured observation of the mobilization and translocation of materials, and served to reveal the speed, direction and magnitude of changes in concentration of certain nutrients, notably magnesium, potassium and calcium.

Consideration of the results obtained for the sampling interval studied in 1954 leads to the following comments in respect of the problem of sampling for diagnosis for adequacy of nutrient supply:

(1) Since the total nitrogen content of the stem-leaf tissues declined continuously, it seems possible that the residual nitrogen in these tissues at the post bloom period may prove to be a measure of the adequacy of supply of this nutrient. The same may be true for total potassium content at the post bloom sampling.

(2) The total phosphorus content proved not to be very variable. Further, the low values obtained for this nutrient, as compared to those reported in the literature for this crop under conditions of adequate phosphorus supply, indicate that sampling of the stem-leaf tissues with analysis for total phosphorus may prove to be a satisfactory guide to adequacy of supply of this nutrient. It is possible, however, that with more ample supply the fluctuations in content of total phosphorus, and perhaps especially of acetic acid-soluble phosphorus, may be greater than were observed under the conditions examined in the present investigation.

The violent changes, in both magnitude and direction, of the magnesium content and of the calcium content, although the changes in the latter were of lesser magnitude, make the time of sampling within the interval examined highly critical, and give rise to corresponding difficulty in establishment of the actual level of and the adequacy of supply of these nutrients. Further, the relation of the concentration of magnesium in the tissue to yield needs to be established.

Since lack of available nitrogen often restricts hay crop production by long-established grass swards and since the phosphorus content found in the samples analysed in 1953 was lower than the average reported for timothy by Beeson, it was decided to examine the effects of nitrogenous and phosphatic fertilization on the crop grown in 1954. The results obtained are recorded in Section II.

SECTION II

EFFECT OF NITROGEN FERTILIZATION ON PLANT COMPOSITION

The application of nitrogen produced no significant change in the moisture content of either stem leaf or corm tissues.

The mean values (four replicates) for the total nitrogen contents of tissues for plants receiving and not receiving nitrogenous fertilizer are presented in Table IX.

TABLE IX

MEAN VALUES FOR THE TOTAL NITROGEN CONTENT (AS % OVEN D.M.) OF STEM-LEAF AND CORM SAMPLES OF TIMOTHY AS EFFECTED BY NITROGEN APPLICATION TO THE SOIL

Date	Stage of Maturity	Total nitrogen content in % +N	No N	Differences
	Prebloom			
25.6.54	2nd internode	1.200	1.012	0.19#
	3rd internode	0.705	0.704	0.001
	Corm	0.174	0.143	0.031#
	Early bloom			
6.7.54	2nd internode	0.857	0.762	0.095#
	3rd internode	0.566	0.542	0.024#
	Corm	0.150	0.129	0.021
	Full bloom			
14.7.54	2nd internode	0.682	0.629	0.053#
	3rd internode	0.426	0.489	0.063#
	Corm	0.195	0.140	0.055#
	Post bloom			
22.7.54	2nd internode	0.707	0.559	0.152#
	3rd internode	0.433	0.325	0.108#
	Corm	0.293	0.139	0.154#

Significant at .05

Inspection of Table IX shows that the trend of change in the total nitrogen content of tissues sampled has not been altered by the application of nitrogen to the soil. The trends are in the same direction for both fertilized and unfertilized plants.

The differences in total nitrogen content attributed to treatment and the significance of these differences are shown in Table IX. The detailed design of statistical analysis has been presented in Appendix D.

The data presented here shows that nitrogen applications to the soil significantly increased the total nitrogen content of the tissues analysed.

The percentage changes in composition of the timothy stem leaf internodes and corms collected in 1954 as a result of nitrogen application to the soil have been presented in Table X.

TABLE X

DIFFERENCES IN COMPOSITION ATTRIBUTED TO FERTILIZATION WITH NITROGEN AND THEIR STATISTICAL SIGNIFICANCE (AS PER CENT CHANGES)

Component	Sampling dates	Second internode	third internode	Corms
Acid extractable carbohydrate	25.6.54	+3	+15*	-16*
	6.7.54	+8*	+26*	+1
	14.7.54	+20**	+17**	+22**
	22.7.54	+7	+17*	+9*
Acetic acid soluble phosphorus	25.6.54	+6	+3	+42*
	6.7.54	+7	+4	+15*
	14.7.54	-8	-9*	-9*
	22.7.54	-14*	-19*	-36*
Total phosphorus	25.6.54	+74**	+0	+8*
	6.7.54	+34**	-22*	+15*
	14.7.54	+21	-4	+13*
	22.7.54	+12	-3*	+15*
Alcohol soluble nitrogen	25.6.54	+5	+8*	+9*
	6.7.54	+6	+30**	+16*
	14.7.54	+39*	+4	+24**
	22.7.54	+21*	+50**	+5
Total magnesium	25.6.54	+1	-9*	-4
	6.7.54	-36**	-39**	-35*
	14.7.54	+26*	+84**	+17*
	22.7.54	+9*	+3	+6
Total potassium	25.6.54	-4	-3	-2
	6.7.54	-4	-12**	-31*
	14.7.54	+4	-5*	+20*
	22.7.54	+11**	+3*	+3
Total calcium	25.6.54	-4	-6	-12
	6.7.54	+7	+16*	-44**
	14.7.54	+9	+3	-25*
	22.7.54	+8	+1	-24*
Total nitrogen	25.6.54	+19*	+0	+21*
	6.7.54	+13*	+4	+15*
	14.7.54	+8*	-14*	+38*
	22.7.54	+31*	+35*	+110**

\+ increase) of the component
\- decrease) due to nitrogen application

*significant at P = 05
** significant at P = 01

Reference to the data presented in Table IX, Appendix C, leads to the following conclusions:

That, except in the case of the element magnesium, and to a lesser extent phosphorus, fertilization with nitrogen had relatively little effect on the seasonal trends in composition of the timothy plant.

The fact that seasonal trends in composition are but little affected by nitrogen fertilization is reassuring from the standpoint of analysis for fertility diagnosis. In particular, the data of Table IX support the suggestion, made earlier, that the post bloom period is a relatively favourable one for analysis for total nitrogen as a guide to sufficiency of supply of this nutrient. At the same time, the data of Table IX, (Appendix C), show that in evaluation of the phosphorus status it is desirable to have information on the nitrogen supply also, similarly for evaluation of the magnesium status.

Consideration of the data presented in Table X leads to the following conclusions:

(1) That fertilization with nitrogen increased both the total nitrogen and the alcohol-soluble nitrogen contents of the tissues examined. The greatest percentage increases in total nitrogen were recorded at the post bloom stage in all three tissues. Increases in alcohol-soluble nitrogen content were especially large in the second internode at the full and post bloom stages, and in the third internode at the early and post bloom periods. Application of nitrogen produced larger changes in the total nitrogen than in the alcohol-soluble nitrogen

contents of the corms,

2. That fertilization with nitrogen had the effect of increasing the extractable carbohydrate content at all stages in both second and third internodes. This probably is a result of increased leaf area. Application of nitrogen markedly decreased the extractable carbohydrate content of the corms at the pre-bloom stage and markedly increased their content of this component at the full bloom stage.

3. That, in comparison with plants not receiving nitrogen, the acetic-acid soluble phosphorus content of the second and third internodes of the fertilized plants was markedly decreased at the post bloom stage. On the other hand, the total phosphorus content of the second internode was increased at all stages, but especially at the pre-bloom period, whereas the total phosphorus in the third node was markedly decreased at the early bloom stage. Relatively, the total phosphorus content of the corms was less affected by nitrogen fertilization than was that of acetic-acid-soluble phosphorus. These observations appear to indicate an effect of nitrogen in increasing the mobility of phosphorus in the plant.

That, in respect of cation concentration (Table IX, Appendix C) the effect of nitrogen fertilization was relatively small for the second and third internodes, whereas depletion of the calcium content of the corms appeared to be favoured; on the other hand, the effect on magnesium was more pronounced, the accumulation of magnesium in the second and third internodes being eliminated and the subsequent depletion of the magnesium content of these tissues being less pronounced when nitrogen was applied.

Odelien (76) has demonstrated different nature of variation in the saccharose content of timothy. He found that at the heading stage, the content of saccharose considerably decreased every year with increasing fertilizer rates. When the cutting was performed at the blooming stage the relation between the fertilizer rate and content of saccharose was not clear. In the first two years he found that the content tended to increase with increasing fertilizer whereas corresponding figures for the last two years of his experiment showed a marked decrease. He analysed the whole plant above ground.

It seems probable that in the early blooming stage when the plant is in its prime vegetative growth, the second internode, as any other part of the plant will tend to accumulate more acid extractable carbohydrate than any other stage. Odelien assumes that when the nitrogen and the mineral supply is optimum the consumption of sugar for the formation of cellulose, other carbohydrates and protein is speeded which is perhaps true and may cause a depletion but the rate of photosynthesis is also speeded up during this period. In the prebloom stage there is a significant increase in soluble phosphorus in the second and the third internode but a decrease in all tissues at the full and post bloom stages.

The total phosphorus content of the second internode and the corm tissues is increased up to the full bloom stage as a result of nitrogen application to the soil. The nature of variation is similar to that of acid extractable carbohydrate.

Significant (+) correlation has been obtained between acid extractable carbohydrate of the second internode and the total phosphorus content in all stages except in the post bloom period. The total phosphorus content (Table IX, Appendix C) of the third internode decreased from early bloom till the post bloom; and this is suggestive of increased mobility of the element. The ratio between the total phosphorus and soluble phosphorus in the untreated plants varies widely (up to 35%) between different dates of sampling whereas this ratio in the treated plants remains fairly constant, throughout the sampling period.

The effect of nitrogenous fertilizer to the soil on the alcohol soluble nitrogen fraction of the stem leaf and corm samples is interesting. This fraction of all tissues analysed was increased. Highly significant (+) correlation was obtained between alcohol-soluble nitrogen fraction and acid extractable carbohydrate of the second internode. Here again reference may be drawn to Odelien (76) who found low sugar content in the seeding stage of the timothy plant but the author believes this is more probable in the full bloom stage. The alcohol soluble nitrogen fraction bears definite correlation (+) with total nitrogen and acid extractable carbohydrate and this may be important in assessing the nutritive status of the timothy plant.

As a result of nitrogen fertilization the magnesium content of the second and third internodal tissues at pre-bloom and the full bloom stage had lessened the content of this element in the tissues and also of corm tissues at the early bloom.

The apparent influence of nitrogen fertilization on the total potassium content of the internodal tissues was relatively small as indicated by data of Table X. However, there is indication of increase in the second internode at post bloom and corm at full bloom with decreases indicated for third internode and corms at the early bloom stages.

The application of nitrogenous fertilizer to the soil does not seem to have much significant effect on the total calcium content of the stem leaf tissues but the same of the corm is considerably decreased, although they are not statistically significant.

The variations as effected by the application of nitrogenous fertilizer to the soil in all the constituents has been found to be significant between sampling dates, i.e. between different stages of maturity, the variation within one sampling is not significant.

Although it appears from the previous discussion that the total potassium content of the stem leaf and partially the corm sample is depressed (in the early stages) as a result of application of nitrogen to the soil, but the total potassium of the whole plant above the ground seems to be more in the samples from nitrogen treated plots. This has been found by analysing the hay samples (Table XI) after harvesting. As such it becomes more clear that the depletion of potassium in the particular parts analysed may be due to translocation which takes place more efficiently as a result of nitrogen application to the soil.

It will further be observed from the analytical data on the hay samples that the whole plant above ground contains much less moisture, nitrogen and phosphorus than the particular parts analysed (the second and third internodes). It further supports the idea that the decrease of total nitrogen in the second internode with the progressive growth of the plant is due more to translocation than dilution because had dilution been the only cause then the total nitrogen content in the hay samples from nitrogen treated plots would have shown a lower value than the control plots. Because if no nitrogen is taken up by the plant as a result of nitrogen application to the soil, and in the meantime the plant is growing in its vegetative size the percentage concentration of the nitrogen in the plant as a whole would have gone down. The analysis of hay further shows that the total phosphorus content in the samples from nitrogen treated plots is much lower than those from the control. The extent of decrease suggests that dilution due to great vegetative growth in the nitrogen treated plots may not be the cause of this low value.

TABLE XI

MEAN VALUES FOR TOTAL NITROGEN, TOTAL PHOSPHORUS (AS % OF OVEN DRY MATTER) AND MOISTURE CONTENT OF THE HAY SAMPLES.

Constituents	Control	s.d.	Nitrogen treated	s.d.
Moisture %	51.85	-	52.85	-
Total Nitrogen	0.468	0.02	0.533	0.017
Total Phosphorus	0.1269	0.004	0.1062	0.004
Total Potassium	1.414	0.017	1.612	0.0037

SECTION III

EFFECT OF POST HARVEST APPLICATION OF PHOSPHORUS TO THE SOIL ON THE CHEMICAL COMPOSITION OF CORMS.

In Table XII the percentage changes in the different constituents obtained by chemical analysis of the corm samples as a result of post-harvest application of non-active phosphorus to the soil has been presented. The data from which the percentages have been calculated have been given in Table X of Appendix C.

TABLE XII

PERCENTAGE CHANGES IN POST HARVEST COMPOSITION OF CORMS ATTRIBUTED TO FERTILIZER TREATMENTS: DIFFERENCE FROM NO TREATMENT AT TWO DATES OF COLLECTION EXPRESSED AS PERCENTAGES (IN 1954).

Components	Nitrogen		Phosphorus		Nitrogen and Phosphorus	
	Sept. 10	Oct. 7	Sept. 10	Oct. 7	Sept. 10	Oct. 7
Acid extract-able Carbohydrate	+23*	+10*	-2.6	-2.2	+8.8*	+8.0*
Total N	+17*	+9**	+10**	-4	-13**	-5
Total P	nil	nil	+7*	+11*	-6	-4
HAc Sol.P	+7*	+1	+17**	+12**	+13**	+6
Total K	+2	+5	+4.6	+2	+3	+2
Total mg	+36**	+36**	+21**	+21**	-6	-6
Total ca	+ 9*	+17**	-7	-10*	-2	+13**

+ increase) of the component due to
− decrease) phosphorus application

Difference significant at P = 05 marked *
at P = 01 marked **

Most of the changes recorded in Table XII are below the level of statistical significance. Only those of considerable magnitude and of consistent direction on the two collection dates will be commented upon.

It is to be noted that, on both autumnal dates of collection, an effect of fertilization with nitrogen in May is indicated. Thus, as found for earlier samplings, corms from plots receiving nitrogen have higher contents of extractable carbohydrate, total nitrogen and magnesium than those from plots which did not receive such fertilization. A tendency toward an increased content of calcium in the nitrogen-treated samples also is to be noted.

Application of superphosphate in August apparently increased both the total and the acetic acid-soluble phosphorus content, the major effect being on the soluble form, which accounted for the total increase in phosphorus at the last date of sampling. The data suggest an increase in magnesium content as another result of this fertilization. The apparent depletions of calcium, nitrogen and extractable carbohydrate, if real, are in the direction to be expected when plants somewhat deficient in phosphorus receive an additional supply of this element, which would favour increased root and shoot growth as compared with plants receiving no additional phosphorus.

The apparent changes in extractable carbohydrate and total nitrogen occurring when both fertilizers are applied are in accord with the effects produced when they are applied singly. In combination, these treatments would be expected to encourage the greatest development of growth, of renewed root and shoot production. It is not surprising, therefore, to find an indication of depletion of total phosphorus content and a lesser increase in acetic acid-soluble phosphorus as compared with corms receiving phosphorus only. The content of magnesium shows the same downward tendency.

It may be noted also (see Table X in Appendix C) that analysis indicated that, regardless of treatment the corm tissues increased in content of total nitrogen, both total and acetic-acid-soluble phosphorus, and of calcium in the interval September 10th to October 7th.

Phosphatic fertilizer effects increasing the total potassium content of the corms. Although experiments of this nature have not been reported bu the exploitation of soil potash reserve with a Calcium - P - legume programme has been suggested by Yoder (123) in Ohio Agricultural Experimental Station. Increasing amounts of phosphatic fertilizer had been found to release more potash from the soil and made available to the plant.

Post harvest application of phosphorus to the soil effects in increasing the total magnesium content of the corms but the increase is not as much as effected by the application of nitrogen to soil. Samples from plots receiving a pretreatment of nitrogen before the application of phosphorus to the soil show the lowest figure for magnesium. The variation of the magnesium content of the corms is practically nil. As opposed to magnesium total potassium content of corms increase due to the application of phosphate to the soil but here also nitrogen pretreatment progressively depresses the uptake of potassium.

Similar responses of various forage grasses and legume seedlings to phosphatic fertilization under greenhouse conditions have been demonstrated by Robinson (89).

He has shown that the application of phosphorus to the soil significantly increases the phosphorus content of the plant, and also the potash content.

The most striking variation as a result of phosphate application to soil seems to occur in the calcium content of the corms. Although application of phosphorus alone seems to have a depressing effect but the pretreatment of nitrogenous fertilizer (NP) has a residual effect in increasing the total calcium content of the corms; nitrogen alone, however, effects this increase more than nitrogen and phosphorus combined.

The effect of nitrogenous fertilizer seems to be hostile in presence of phosphatic fertilizer. That nitrogenous fertilizer in the soil effects in depressing the uptake of phosphorus by the timothy corms has been demonstrated by the application of radioactive phosphorus (KH_2PO_4) to the soil.

UPTAKE OF RADIOACTIVE PHOSPHATE

As commented earlier, the 1954 growing season was characterized by high rainfall throughout. Radioactive phosphate was applied August 10, 1954. For the months of August and September, 1954, the total rainfall amounted to 8.33 inches as compared to a 7-year average of 6.6 inches for these two months. This relatively heavy precipitation apparently favoured rapid and relatively deep penetration of the soluble salt potassium acid phosphate.

Owing to the extreme wetness of the season less than 50% of the active phosphate remained in the top inch of the soil. This was true for all plots. Soil samples were collected by a soil borer at appropriate depth and the activity of each inch was determined by a portable Geiger counter with an ear phone attached to it for recording the counts. Complete data on the activity up to three inches of the soil treated with radioactive phosphorus have been given in Appendix E.

Counts on the old corms on all the plots on all the three different samples were negligibly small. This can be partially accounted for by the fact that most of the active phosphorus was leached down by the rainfall below the limit of reach by the roots. It may further be explained that the failure of the old corms to accumulate radioactive phosphorus could be either due to the inactivity of their root systems or due to rapid translocation of any radio phosphorus from the old to the new corms. Active phosphorus, however, did appear in the new growth of stem leaf and corms.

TABLE XIII

EFFECT OF NITROGEN ON THE PHOSPHORUS UPTAKE

BY THE NEW GROWTH OF TIMOTHY STEM LEAF TISSUE

PLUS CORMS

Date	Plot No.	Dry weight of sample in gms.	Total counts	Cts/ min/gm.	Average cts/ min/gm.
	1C#	2.87	1463	510	
	2C	2.50	1282	513	551
August	7C	2.12	1302	614	
31	8C	2.49	1414	568	
1954	3N##	2.92	575	197	
	4N	3.10	527	169	222
	5N	3.40	561	165	
	6N	4.12	1473	357	
	1C	2.40	1544	643	
	2C	2.10	1499	714	719
September	7C	2.00	1474	737	
9	8C	2.09	1634	782	
1954	3N	2.51	1138	453	
	4N	3.00	1132	377	367
	5N	3.12	838	268	
	6N	4.10	1509	368	

Control Plots

Plots receiving nitrogen at the rate of 50 lbs. of $NH_4 NO_3$ per acre on 15.5.54.

Table XIII giving the results on two different dates shows the effect of nitrogen on the uptake of phosphorus by the new growth (i.e. the new corms and their stem leaves) of the timothy plant.

It is evident from Table XIII that when both nitrogen and phosphorus were applied (i.e. phosphorus applied to plants which had and plants which had not received an earlier fertilization with nitrogen) the uptake of phosphorus is markedly depressed when nitrogen had been applied. The effect of nitrogen would help making more shoot growth confirmed that. This increased shoot growth may partially be responsible for depressed phosphorus uptake when nitrogen is applied. However, further investigation would be desirable before definite comment on this subject is made. There is a time factor entering in the depressed uptake of phosphorus over the period of experiment: over the period of experiment there was a 30% increase in the phosphorus uptake on the control plots, but a 60% increase on the nitrogen treated plots. It will appear that while nitrogen depresses the uptake of phosphorus, its retarding effect decreases with time. Thus the ratio counts/min./gm. for control plots versus N-treated plots on August 31st was 55/22 (=2.5) on September 9th it was 72/37 (=1.9).

SUMMARY

Stem leaf internodal samples and corm tissues of the timothy plant have been analysed at different stages of maturity in 1953 and 1954 to investigate the variations in the composition.

Standard procedures of chemical analysis, with slight modifications in some cases, have been adopted in determining the nitrogen, acid extracted carbohydrate, phosphorus, magnesium, potassium and calcium content of the tissues.

In 1953, stem-leaf tissue of the second internode (from the top) was sampled during the growing season and, after harvesting of the hay, corms were also collected for analysis. In 1954, both second and third internodes (from the top) were sampled and corms were also collected contemporaneously. Post-harvest corms also were analysed.

Studies were made in 1954 on the effect of nitrogenous fertilizer on the composition of the plant, and also of the post-harvest application of phosphorus (active and non-active) on the composition of the corms. Thus the work may be divided into three sections:

SECTION I. UNFERTILIZED PLANT COMPOSITION.

The results which have been obtained regarding the composition of the samples analysed may be summarized as follows:

(a) A gradual decrease of the moisture content in all the stem leaf internodal samples has been observed with the progressive maturity of the plants. The variations in the moisture content of the corms are irregular and no definite interpretation can be made.

(b) As the plant grows the dry weight of the corms decreases and that of stem leaf internodes increases. These variations are significant in all stages of corm samples except in the early bloom stage, and highly significant in all periods of growth in case of the stem leaf internodal tissues.

(c) In 1953, the second internode had the highest percentage nitrogen during the blooming period and the post bloom, the lowest, whereas in 1954 there was a continuous depletion of nitrogen from both second and third internode. Total nitrogen in the corms in both the years showed a percentage increase although there was a decrease in the Sept. 18 (1953) figure. The statistical significance of these variations has been shown. Consideration of the dry weight figures for stem leaf and corm samples and the variations in the content of nitrogen in them (1954) suggests that at least a part of nitrogen is being translocated from the corms towards the tops.

(d) In 1953 the percentage acid extractable carbohydrate content of the second internode decreased continuously from the prebloom to the post bloom period, but in 1954 these variations were irregular both in the second and third internode.

Determination of the one per cent oxalic acid extractable carbohydrate was also made after exhaustive extraction of the stem leaf tissues with 80% aqueous ethanol. The difference between this fraction and the acid extractable carbohydrate was considered to represent the simple sugar content.

Simple sugars have been found to decrease progressively in the stem leaf tissues in all stages except in the early growing stage. Chromatographic investigation showed the principal simple sugar lost was sucrose.

(e) The nature of variation in the soluble phosphorus content of internodal tissues and corm samples was different in 1953 and 1954. The content of soluble phosphorus in the third internode is relatively constant during the reproductive phase.

The total phosphorus content of stem leaf samples differed between the two years, - 1953 samples having a higher content than 1954 samples, but in general the phosphorus content was low. Post harvest corms (as on Sept. 18, 1953 and Oct. 7, 1954) had the highest content of phosphorus.

(f) Total magnesium content varied greatly in two years. In 1953 the content of magnesium was mugh higher in the post bloom than during the same period in 1954.

Magnesium has been found to be accumulated in the stem leaf tissues at the early bloom in 1954 with subsequent rapid depletion. The data indicated a rapid upward translocation of the element during the flowering and seed setting stage; the second internode samples of 1953 crop showed both higher nitrogen and higher magnesium status than those of the 1954 crop suggesting a direct relation of magnesium with the nitrogen status of the plant during the prebloom and early bloom periods.

(g) In 1954 the percentage potassium content of internodal tissues decreased with maturity but in 1953 the trend seems to be somewhat different although the post bloom period had the lowest content of this element in both the years. Similar tendencies have been shown by the corms. These variations when considered in the light of the dry weight changes of the corm tissues are not significant.

(h) Total calcium content of the internodes decreases in the early bloom stage and is followed by an increase in the post bloom stage.

SECTION II. EFFECT OF NITROGENOUS FERTILIZER ON THE PLANT COMPOSITION

As a result of nitrogen application to the soil, significant variations in all the constituents analysed were observed in almost all periods of growth of all the tissues except the total calcium content of the second internode.

(a) The simple carbohydrate content increased significantly in all cases except in the prebloom stage of the second internode and corm in which the simple carbohydrate is significantly less.

(b) The data presented show that nitrogen content of all the tissues analysed increased significantly as a result of nitrogen application to the soil.

(c) The dry weight of the stem leaf tissues and corms do not change significantly due to nitrogen application though there is a general tendency to do so as shown by the percentage increase in every case.

(d) The alcohol soluble nitrogen fraction of all the tissues increased due to nitrogen application to the soil and this fraction in the second internode bears a correlation (+) with the total nitrogen and acid extracted carbohydrate.

(e) In the pre-bloom stage there is a significant increase in soluble phosphorus of stem leaf tissues but a decrease in all tissues at full and post bloom stages.

(f) The total phosphorus of stem leaf of second internode and corm tissue increases and this fraction of the second internode bears a correlation (+) with the acid extracted carbohydrate up to the full bloom stage.

(g) The magnesium content of internodal tissues lessens in all the tissues at early bloom period and indications of less depletion of all the tissues in full bloom stage have been observed.

(h) The potassium content of all the tissues was less at the pre-bloom and the early bloom stages and increased at the other stages.

(i) Calcium content of the stem leaf tissues does not vary significantly whereas the same in the corms is decreased considerably due to nitrogen application to the soil.

SECTION III

EFFECT OF POST HARVEST APPLICATION OF PHOSPHORUS ON THE PLANT COMPOSITION

Phosphorus alone depresses the accumulation of acid extracted carbohydrate but a pretreatment with nitrogenous fertilizer minimises this effect to a considerable extent.

Application of superphosphate in August apparently increased both the total and the acetic acid-soluble phosphorus content, the major effect being on the soluble form, which accounted for the total increase in phosphorus at the last date of sampling. The data suggest an increase in magnesium content as another result of this fertilization. The apparent depletions of calcium, nitrogen and extractable carbohydrate, if real, are in the direction to be expected when plants somewhat deficient in phosphorus receive an additional supply of this element, which would favour increased root and shoot growth as compared with plants receiving no additional phosphorus.

CONCLUSIONS

(1) Moisture content of the stem leaf tissues sampled varied (decreased) with stage of maturity and with seasonal climatic conditions, these seasonal decreases apparently being greater in the season of higher rainfall (1954). The moisture changes of corm tissues were so variable as to be very difficult to interpret.

(2) Table VI particularly data for 1954 suggests that second and third internodal tissues were continuously depleted of nitrogen during the blossoming period. Corm tissues of 1954 samplings, July 14-22, lost nitrogen, and dry weight with no change in nitrogen percentage; as such there was a loss of nitrogen from the corms.

Table VI further shows that the changes in percentage total nitrogen between sampling dates were significant in both years and for all tissues sampled except corms sampled on July 14 and 22, 1954.

(3) Table II, (Appendix C), shows that percentage changes in acid extractable carbohydrate between sampling dates were significant at 0.05 for most tissues in both years; exceptions were:

Corms (1953), July 25-Sept. 10 samplings
Stem leaf (1954), July 14-July 22 samplings
Corms (1954), Sept. 10-Oct. 7 samplings.

Tables II and III of Appendix C indicate that decrease of carbohydrate is due partly to the loss with increasing maturity of the

80 per cent ethanol soluble fraction. Chromatographic investigation of this fraction showed that sucrose was the principal sugar lost. Chromatographic study also showed that the fraction insoluble in 80 per cent ethanol but soluble in water and hydrolysed by refluxing with 1 per cent $H_2 C_2O_4$ for one hour consisted of fructosans.

(4) Differences in the content of HAc. soluble phosphorus in the second internode stem leaf tissues were observed in the two seasons. There was evidence of translocation of HAc. soluble phosphorus from second node to inflorescence. The behaviour of this fraction differed from that of total nitrogen and of simple sugar plus hydrolysable carbohydrate in that the percentage of HAc. soluble phosphorus was much more constant throughout the flowering period. The relative constancy (no significant change between sampling dates) during the early to post bloom period of the amount of this fraction in the third internode tissue (1954) suggests continuous uptake from the soil during this interval or relative immobility of the phosphorus.

Statistically significant changes in percentage of HAc. soluble phosphorus between sampling dates were found in case of stem leaf tissues of the second internode in bloom to post bloom stage in 1953 (+ve), also in the post-hay harvest corms in 1953 and 1954 (differing in sign 1953 and 1954).

(5) Total phosphorus percentages differed in the two seasons being somewhat higher in the second internode stem leaf tissues in 1953 than in 1954. This well may be

attributed to a dilution effect, the plant being larger in 1954 than in 1953 (visual observation, no experimental proof available). The difference in percentage total phosphorus between sampling dates were significant for the second internode tissues in 1953 and for corm tissues in both years, but not for either second or third internode tissues in 1954. In general the total phosphorus content of the stem leaf tissues were low and suggested the need for phosphate fertilization.

(6) Total magnesium content of all tissues analysed showed wide differences in 1954 from those values found in 1953. The differences observed suggested greater mobility of magnesium within the plant in 1954. The literature records that low nitrogen status may be accompanied by low magnesium status. It may be significant that the plants of 1953 crop were higher in both nitrogen and magnesium than those of the 1954 crops.

(7) The total potassium content of the tissues sampled indicated an adequate supply of this element in both years. Statistically significant decreases in the percentages of potassium in the stem leaf tissues of both the second and the third internodes occurred in 1954 suggesting translocation of this element to the inflorescence during the blooming period.

(8) The data for total calcium suggest translocation to inflorescence from the second internode in the period pre-bloom to early bloom in both years, followed by an apparent increase in both years (greater in 1954) for second internode and for third internode in 1954.

(9) In the 1954 season nitrogen fertilization increased the total nitrogen content of all tissue analysed but had no significant effect on the trend of seasonal change in the nitrogen contents of these tissues.

The application of nitrogenous fertilizers in this season produced significant changes (as compared to plants receiving no nitrogen fertilizer) in content or behaviour of tissue components which are summarized below:

(a) The alcohol-soluble nitrogen content of all tissues was greater on all sampling dates.

(b) The acid extractable carbohydrate content of all tissues was greater, except for corms at the pre-bloom stage in which the acid extractable carbohydrate content was significantly low.

The higher acid extractable carbohydrate has presumably resulted from the increase in leaf area brought about by nitrogenous fertilization.

(c) HAc. soluble phosphorus was greater in the second internode tissue and the corm tissue at the pre-bloom and early bloom stages and lower at the full and post bloom stages. These results suggest that nitrogen fertilization increased the mobility of phosphorus.

The total phosphorus content of the second internode and the corm tissues showed consistent increase at all stages while third internode showed consistent decrease. This suggests greater uptake of phosphorus by the plants. The fact that the total phosphorus content of the third internode tissues was decreased at the early and the post bloom periods is suggestive of increased mobility of this element.

(d) The fact that nitrogen fertiliaation increased the magnesium content of the second and the third internodal tissues at the full bloom periods and lessened the content of this element in all the tissues at the early bloom stage suggests an effect on the mobility of magnesium as well as of phosphorus.

(e) Since the potassium content of all tissues was less at the pre-bloom and early bloom stages and of the tissues of the third internode at full bloom as well, it appears that the mobility of this element also was increased.

The fact that the potassium content of the tissues of the second internode and of the corms was increased at the full bloom stage and that the same was true for those of the third internode and of the corms at the post bloom suggests that the potassium supplying power of the soil was satisfactory and that nitrogen fertilization favoured uptake of this element.

(10) Application of superphosphate (SP) after harvesting the hay produced the following significant changes in percentage composition of the corm tissues:

(a) **acid extracted carbohydrate** was decreased by SP without earlier N fertilization, increased by SP with earlier N fertilization.

(b) HAc. soluble phosphorus was increased by SP in case of both untreated and N-fertilized areas.

(c) The total phosphorus content of the corms decreased in the order, phosphorus alone $>$ nitrogen alone $>$ nitrogen and phosphorus, and in case of magnesium the order is nitrogen alone $>$ phosphorus alone $>$ nitrogen and phosphorus.

(d) The percentage content of potassium was increased by use of SP alone and the pretreatment of nitrogen had the greatest effect in increasing the calcium content.

(11) The following conclusions respecting the sampling of the various tissues analysed, for use in the diagnosis of nutrient status, appears, on the basis of the percentage composition results so far obtained, to be justified, namely:

(a) Assessment of nitrogen status by analysis of either second or third internodal tissues would appear in view of the large seasonal differences observed, to be difficult.

It is possible, however, that measurement of alcohol-soluble nitrogen might be more useful, since it was found that fertilization with nitrogen increased this fraction significantly.

(b) The relative constancy during the flowering period of both the HAc. soluble and the total phosphorus contents of the third internode suggests that sampling and analysis of this part of the plant could provide useful information on the phosphorus status. However, the results obtained with nitrogen-fertilized timothy indicate that the phosphorus status cannot be assessed without consideration of the nitrogen status.

(c) Since the magnesium content of the internodal tissues examined was found to be relatively constant in the later part of the blooming period, it appears that samples of the tissues taken at that time would give useful information. The results obtained for nitrogen-fertilized timothy indicate that the nitrogen status needs consideration in the assessment of magnesium status as well as of phosphorus status.

(d) The relatively low percentage of potassium in the internodal tissues towards the end of the blooming period suggests that sampling of the tissues at this time might provide useful information on the potassium status.

Similarly, the relatively low percentage calcium in the tissues of the second and the third internodes at early bloom suggests sampling of these tissues at this time. The relatively rapid increase thereafter suggests that it would be advisable to sample twice.

(e) The information so far obtained suggests that the carbohydrate status as well as the nitrogen status could best be assessed by measurement of the fraction soluble in 80% alcohol.

CLAIMS FOR CONTRIBUTION TO KNOWLEDGE

A search of the literature indicates that the present investigation is original in the following respects:

(a) In that it constitutes a first attempt to estimate the variations in composition of the timothy plant during the reproductive phase making use of simultaneously collected stem-leaf and corm samples;

(b) In that it provides, for the first time, detailed information on the cationic composition and the acetic acid soluble phosphorus content of the corm tissues;

(c) In that it provides, for the first time, information of the type required for (1), the selection of the part of the timothy plant to sample and (2), the selection of the most appropriate time in the life cycle of the plant to collect samples when the objective is the assessment of the adequacy of supply of nutrients such as nitrogen, phosphorus, potassium, calcium and magnesium; and

(d) In that it provides new information relative to the mobility of nutrients within the plant during the reproductive phase.

The results obtained in this investigation also provide evidence of a very limited capacity on the part of the perennial grass timothy to make use, during the late summer and early autumn, of the phosphorus of either superphosphate or potassium acid phosphate applied to the sod following harvest of the hay crop.

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APPENDIX A

DESCRIPTION OF THE SOILS

Chateauguay series

Chateauguay soils are smooth and generally undulating, although gently rolling areas also occur. Slopes are commonly of 3 to 4 per cent, but some slopes are up to 7 or 8 per cent. Considerable sheet and rill erosion has been noticed on some of the steepest slopes.

The parent material is a clay loam deposited over calcareous till, to a thickness varying from a few inches to about 4 ft. but generally to about 2½ ft. The texture of this material is not uniform but varies from a clay loam to a sandy clay loam, with pockets of clay and of sandy loam. Only occasional stones are seen on and through the solum.

The Chateauguay soils are moderately well drained and they have a good moisture holding capacity.

The Chateauguay soil is composed of the clay loam, the shallow clay loam and the loam. The clay loam is the common type and it is described below.

Horizon depth:

(Ao) 0-7" Very dark grayish brown clay loam, medium granular structure friable, slightly sticky and plastic, pH 6.2.

(A_1) 7-18" Dark grayish brown to brown, clay loam to clay with spots and streaks of grayish brown of lighter texture, slightly sticky and plastic, slightly firm pH 6.2.

(A_2) 18-30" Brown to grayish brown clay loam to clay with some spots of lighter and pale brown colour; the colour is not uniform and also includes some dark brown; dark root channels; very faint fine mottling coarse nuciform structure; slightly firm, slightly plastic and sticky. pH 6.4.

(B_1) 30-40" Dark brown with some brown clay to clay loam, also dark grayish brown colour; texture variable but as a whole heavier than above; fragmental or blocky macrostructure breaking into fine nuciform microstructure, not well defined as a whole; slightly to moderately plastic and sticky; firm, pH 6.6.

(CD) 40" Brownish gray calcareous gravelly clay loam to gravelly loam till; stones; very firm; massive or fragmental structure, effervescence, pH 7.8. There are few stone fragments and some mica particles through the solum. The stone fragments are greatly of limestone and may effervesce if not completely weathered.

Macdonald Soils

The Macdonald soils are found associated with the Chateauguay soils, on land which is almost level or slightly depressed. The external drainage is slow while the internal drainage is moderate. The profile has developed from alluvio-lacustrine clay loam material deposited over calcareous till to an average depth of $2\frac{1}{2}$ ft. Only few scattered stones are found on the soil surface or through the profile. There is no danger of accelerated erosion on the soils but they may suffer from accumulation of eroded material coming from surrounding slopes.

The clay loam which is described below is the dominant soil in the Macdonald series, however, one subtype has been set up where the subsoil contains reddish clay instead of the usual grayish clay.

Horizon Depth:

(A_0) 0-6" Dark brown clay loam to clay, medium granular and nuciform structure, firm, moderately to slighly plastic and sticky,,pH 5.8.

(B_1) 6-14" Dark grayish brown clay with low contrast fine mottling of grayish loam and dark yellowish brown and some streaks or spots of gray sandy materials; nuciform structure moderately developed; firm plastic and sticky; pH 6.3.

(B_2) 14-22" Dark grayish brown clay loam to clay; medium size and moderate contrast mottling of dark yellowish brown; sandy streaks or patches of gray and ocre weathered stones; many stone fragments partly decomposed, fine nuciform structure, slightly firm, slightly to moderately plastic and sticky, pH 6.8.

(CD) 22" Reworked clay loam till of variable colour, dark yellowish brown dominant with spots of light gray, brownish yellow and very dark gray, numerous stone fragments, many being partly decomposed, massive or slightly developed nuciform structure; firm; slightly plastic; generally containing more than 20% of gravel; effervesces with acid; pH 7.1 to 7.5.

APPENDIX B

RAINFALL, AVERAGE TEMPERATURE AND HOURS OF SUNSHINE

BETWEEN MAY I AND JULY 31, 1953

Dates	Rainfall in inches P.M.	A.M.	Mean for 7 yrs. in inches	Date of sampling	Average temp. during the month	Hours of sunlight
1.5.53		.32	May			Total for May '53 = 235.8
2.5.53		.16		No sampling		
5.5.53	.08		2.98		56.26	
12.5.53	.05					
13.5.53	.18					
14.5.53		1.45				Mean for 79 yrs. = 206.1
18.5.53		.88				
22.5.53	.05	.35				
4.6.53	.23					Total for June '53 = 274.9
5.6.53	.58		June			
6.6.53	.19	.17				
9.6.53	.08	1.15	3.30	24.6.53		Mean for 79 yrs. = 226.9
26.6.53		.62			63.84	
28.6.53				30.6.53		From June 20-30=76.4
2.7.53	.27					Total for July = 258.0
6.7.53	.65	.66	July			
7.7.53	.02					
10.7.53	.17		4.22			Mean for 79 yrs. = 244.5
13.7.53	.18					
24.7.53	.39	.35		7.7.53		
26.7.53	.08			10.7.53	67.37	From July 1-6 = 62.0
27.7.53		.27				
29.7.53	.10			25.7.53		From July 7-14 = 81.1
31.7.53		.03				From July 15-22= 69.9

RAINFALL, AVERAGE TEMPERATURE AND HOURS Of SUNSHINE

BETWEEN AUGUST I & OCTOBER 31, 1953

DAtes	Rainfall in inches P.M.	A.M.	Mean for 7 yrs. in inches	Date of sampling	Average temp. during the month	Hours of sunshine
4.8.53	1.35		August	12.8.53		Total for August = 261.9
10.8.53		.32			62.34	Mean values for 79 yrs. = 226.2
24.8.53	.25		3.53	31.8.53		
4.9.53		.35	Sept.			Total for September = 172.9
5.9.53	.90					
7. 9.53		.35	3.07			
12.9.53	.25					
13.9.53	.25				55.30	Mean for 79 yrs. = 174.6
16.9.53	.06	.16				
18.9.53		.08		18.9.53		
27.9.53		.58				
7.10.53	.80		Oct.			Total for October = 159.6
9.10.53		.05			46.98	
19.10.53		.04	2.87			Mean for 79 yrs. = 139.5

RAINFALL, AVERAGE TEMPERATURE AND HOURS OF SUNSHINE BETWEEN MAY I AND JULY 31, 1954.

Dates	Rainfall in inches P.M.	A.M.	Mean for 7 yrs. in inches	Date of sampling	Average temp. during the month	Hours of sunlight
1.5.54	.07		May			Total for
2.5.54		.47				May =
3.5.54	.34	1.50	2.98			187.2
4.5.54		.07			52.44	
6.5.54	.29					Mean for
8.5.54	.65			No		80 years
9.5.54	.54			sampling		= 206.1
11.5.54	.43					
22.5.54		.58				
28.5.54	.26					
29.5.54	.06					
1.6.54	.47		June			Total for
2.6.54	.80					June =
3.6.54	.04		3.20			172.1
4.6.54		.14				Mean for
5.6.54		.07				80 years
6.6.54	.70				63.08	= 226.5
14.6.54		.14				From the
15.6.54		.18				20th to
20.6.54		.14				30th June
26.6.54	1.10			25.6.53		= 44.2
27.6.54		.25				
1.7.54	.[illegible]	.07	July			Total for
2.7.54	.02					July =
6.7.54	.36		4.22	6.7.53		247
8.7.54	.16					
12.7.54	.08			14.7.53	65.87	Mean for
16.7.54		.01		22.7.53		80 years
20.7.54	.20					= 244.6
21.7.54	.21			26.7.53		
23.7.54	.15					From the
24.7.54		..11				1st to
25.7.54	.11					6th July
30.7.54	.03	.31				= 52.3

RAINFALL, AVERAGE TEMPERATURE AND HOURS OF SUNSHINE

BETWEEN AUGUST I AND OCTOBER 31, 1954

Dates	Rainfall in inches P.M.	A.M.	Mean for 7 yrs. in inches	Date of sampling	Average temp. during the month	Hours of sunlight
1.8.54	.44	.[illegible]	Aug.			Total for
4.8.54		.19				August =
5.8.54		.22	3.53			246.6
6.8.54	.13	.28			64.88	
11.8.54	.01	.27				Mean for
16.8.54	.70			28.8.54		80 yrs. =
25.8.54	.27					226.2
30.8.54	.57	.55				
1.9.54		1.48	Sept.			Total for
3.9.54		.23				September
7.9.54	.24	.20	3.07			= 126.7
13.9.54	.45	.40		9.9.54		
16.9.54	.26				56.4	Mean for
19.9.54	.63			10.9.54		80 yrs. =
22.9.54		.58				174.2
23.9.54	.02					
26.9.54	.37					
30.9.54	.40	.50				
1.10.54		1.44	Oct.			Total for
2.10.54		.03				October =
3.10.54	.35		2.87	6.10.54		125.6
4.10.54		.25		7.10.54		
6.10.54	.25					Mean for
9.10.54	.25				48.98	80 yrs. =
11.10.54	.75					128.4
12.10.54	.30					
14.10.54	.13					
15.10.54	.02					
18.10.54	.19					
26.10.54		.25				
27.10.54	.32					

APPENDIX C

TABLE I

MEAN VALUES AS PER CENT OF OVEN DRY WEIGHT AND STANDARD DEVIATION FOR THE TOTAL NITROGEN CONTENT OF STEM LEAF TISSUE OF THE INTERNODE BELOW THE INFLORESCENCE AND CORMS

1953 samples			1954 samples		
Stages and dates of sampling	Mean #1 %	S.D.	Stages and dates of sampling	Mean #2 %	S.D.
2nd internode			2nd internode		
(1) Prebloom 24.6.53	0.883	0.069	(1) Prebloom 25.6.54	1.012	0.087
(2) Early bloom 30.6.53	1.140	0.064	(2) Early bloom 6.7.54	0.762	0.098
(3)	-	-	(3) Full bloom 14.7.54	0.624	0.039
(4) Post bloom 7.7.53	0.882	0.019	(4) Post bloom 22.7.54	0.559	0.057
3rd internode			3rd internode		
(1) Prebloom	-	-	(1) Prebloom 25.6.54	0.705	0.012
(2) Early bloom	-	-	(2) Early bloom 6.7.54	0.542	0.018
(3) Full bloom	-	-	(3) Full bloom 14.7.54	0.489	0.013
(4) Post bloom	-	-	(4) Post bloom 22.7.54	0.325	0.041
Corms			Corms		
(1) July 10	0.256	0.02	(1) July 14	0.140	0.0066
(2) July 25	0.417	0.0146	(2) July 22	0.139	0.0176
(3) Sept.18	0.358	0.018	(3) Sept.10	0.218	0
			(4) Oct. 7	0.3472	0

#1 mean of 9 samples
2 mean of 4 samples

TABLE II

MEAN VALUES (AS PER CENT OF OVEN D.M.) FOR THE SIMPLE SUGAR PLUS HYDROLYSABLE CARBOHYDRATE CONTENT OF THE TIMOTHY INTERNODES AND CORMS AT DIFFERENT STAGES OF MATURITY IN 1953 AND 1954.

1953 samples			1954 samples		
Stage	Mean #1 %	S.D.	Stage	Mean #2 %	S.D.
2nd internode					
(1) Prebloom	15.58	0.37	(1)	8.76	0.84
(2) Early bloom	11.69	0.50	(2)	11.21	0.69
(3) Full bloom			(3)	8.78	0.12
(4) Post bloom	10.89	0.65	(4)	9.00	0.066
3rd internode					
(1) Prebloom			(1)	8.65	0.62
(2) Early bloom			(2)	12.59	0.89
(3) Full bloom			(3)	11.28	0.47
(4) Post bloom			(4)	10.64	0.38
Corm					
(1) July 10	61.13	1.37	(1) July 14	57.65	0.437
(2) July 25	59.94	0.781	(2) July 22	43.5	0
(3) Sept.18	63.19	0.046	(3) Sept.10	58.75	0
			(4) Oct. 7	58.94	0.63

#I mean of 9 samples
#2 mean of 4 samples

TABLE III

MEAN VALUES FOR ALCOHOL INSOLUBLE ACID HYDROLYSABLE CARBOHYDRATE (AS PER CENT OF OVEN DRY WEIGHT).

1953 sample		1954 sample	
Stages and date of sampling	Mean #1 %	Stages and date of sampling	Mean #2 %
2nd internode		2nd internode	
(1) Prebloom 24.6.53	5.78	(1) Prebloom 25.6.54	6.49
(2) Early bloom 30.6.53	4.27	(2) Early bloom 6.7.54	8.68
(3) Post bloom 7.7.53	4.07	(3) Full bloom 14.7.54	7.44
		(4) Post bloom 22.7.54	9.04
3rd internode		3rd internode	
		(1) Prebloom 25.6.54	5.87
		(2) Early bloom 6.7.54	8.60
		(3) Full bloom 14.7.54	10.66
		(4) Post bloom 22.7.54	10.53
Corms		Corms	
(1) July 10	55.42	(1) July 25	44.60
(2) July 25	58.77	(2) July 6	54.66
(3) Sept. 18	61.96	(3) July 14	50.85
		(4) July 22	43.60

#1 mean of 9 samples

#2 mean of 4 samples

TABLE IV

MEAN VALUES (AS PER CENT OF OVEN DRY WEIGHT) FOR THE 2% ACETIC ACID SOLUBLE PHOSPHORUS OF THE TIMOTHY INTERNODES AND CORMS

Stage	1953 Mean #1	S.D.		1954 Mean #2	S.D.
2nd internode					
(1) Prebloom	0.0745	0.00091		0.0856	0.0015
(2) Early bloom	0.0746	0.0096		0.0911	0.0033
(3) Full bloom				0.0746	0.0029
(4) Post bloom	0.0874	0.00074		0.0728	0.0074
3rd internode					
(1) Prebloom				0.0720	0.0198
(2) Early bloom				0.0838	0.1143
(3) Full bloom				0.0869	0.0019
(4) Post bloom				0.0821	0.0046
Corms			June 25	0.0895	
			July 6	0.1278	
(1) July 10	0.1225	0.00045	(1) July 14	0.1171	0.0013
(2) July 25	0.1320	0.00063	(2) July 22	0.1148	0.0028
(3) Sept.18	0.1190	0.0024	(3) July 10	0.1011	0.00077
			(4) Oct. 7	0.1141	0

#1 mean of 9 samples

#2 mean of 4 samples

TABLE V

MEAN VALUES (AS PER CENT OF OVEN D. M.) FOR
THE TOTAL PHOSPHORUS CONTENT OF THE TIMOTHY
INTERNODES AND CORMS

Stage	1953 Mean % #1	S.D.		1954 Mean % #2	S.D.
2nd internode					
(1) Prebloom	0.1368	0.0023		0.111	0.0089
(2) Early bloom	0.1287	0.0012		0.117	0.0069
(3) Full bloom				0.125	0.00136
(4) Post bloom	0.1300	0.00079		0.127	0.00274
3rd internode					
(1) Prebloom				0.122	0.009
(2) Early bloom				0.148	0.0088
(3) Full bloom				0.139	0.00098
(4) Post bloom				0.134	0.00121
Corms			June 25 July 6	0.1180 0.1310	
(1) July 10	0.1432	0.0025	(1) July 14	0.1644	0.0018
(2) July 25	0.1715	0.0017	(2) July 22	0.1609	0.0016
(3) Sept.18	0.1946	0.0017	(3) Sept.10	0.1647	0.0
			(4) Oct. 7	0.1956	0.0022

#1 mean of 9 samples
#2 mean of 4 samples

TABLE VI

MEAN VALUES (AS PER CENT OF THE OVEN D.M.)

FOR THE TOTAL MAGNESIUM CONTENT OF THE TIMOTHY

INTERNODES AND CORMS

Stage	1953 Mean #1	1953 S.D.		1954 Mean #2	1954 S.D.
2nd internode					
(1) Prebloom	0.115	0.0019		0.197	0.0023
(2) Early bloom	0.119	0.011		0.305	0.0117
(3) Full bloom				0.053	0.004
(4) Post bloom	0.132	0.0079		0.053	0.0025
3rd internode					
(1) Prebloom				0.159	0.014
(2) Early bloom				0.248	0.0065
(3) Full bloom				0.031	0.005
(4) Post bloom				0.039	0.0051
Corms			June 25	0.009	
			July 6	0.010	
(1) July 10	0.084	0.00085	July 14	0.024	0.006
(2) July 25	0.099	0.0016	July 22	0.033	0.0029
(3) Sept.18	0.102	0.004	Sept.10	0.03	0.0028
			Oct. 7	0.03	0.0028

#1 mean of 9 samples

#2 mean of 4 samples.

TABLE VII

MEAN VALUES (AS PER CENT OF OVEN D.M.) FOR THE TOTAL POTASSIUM CONTENT OF THE TIMOTHY INTERNODES AND CORMS

Stage	1953 Mean % #1	1953 S.D.		1954 Mean % #2	1954 S.D.
2nd internode					
(1) Prebloom	2.507	0.068		2.765	0.053
(2) Early bloom	2.570	0.074		2.690	0.08
(3) Full bloom				1.677	0.014
(4) Post bloom	2.400	0.076		1.455	0.011
3rd internode					
(1) Prebloom				2.633	0.028
(2) Early bloom				2.458	0.055
(3) Full bloom				1.486	0.0397
(4) Post bloom				1.287	0.021
			June 25	1.158	
Corms			July 6	1.1240	
(1) July 10	1.503	0.054	(1) July 14	1.164	0.006
(2) July 25	1.630	0.055	(2) July 22	1.164	0.006
(3) Sept.18	1.102	0.021	(3) Sept.10	1.083	0.006
			(4) Oct. 7	1.083	0.006

#1 mean of 9 samples

#2 mean of 4 samples

TABLE VIII

MEAN VALUES (AS PER CENT OF OVEN D.M.) FOR THE TOTAL CALCIUM CONTENT OF THE TIMOTHY INTERNODES AND CORMS

Stage	1953 Mean #1	1953 S.D.		1954 Mean #2	1954 S.D.
2nd internode					
(1) Prebloom	0.240	0.0033		0.290	0.0027
(2) Early bloom	0.230	0.0063		0.267	0.0051
(3) Full bloom				0.362	0.0029
(4) Post bloom	0.243	0.0044		0.405	0.0073
3rd internode					
(1) Prebloom				0.310	0.0033
(2) Early bloom				0.223	0.0166
(3) Full bloom				0.323	0.0053
(4) Post bloom				0.355	0.0031
			June 25	0.128	
Corms			July 6	0.189	
(1) July 10	0.133	0.005	(1) July 14	0.282	0.004
(2) July 25	0.141	0.0046	(2) July 22	0.312	0.0019
(3) Sept.18	0.118	0.0018	(3) Sept.10	0.315	0
			(4) Oct. 7	0.369	0.0017

#I mean of 9 samples
#2 mean of 4 samples

TABLE IX

MEAN VALUES# (AS % OF OVEN D.M.)

VARIATION OF DIFFERENT CONSTITUENTS

DUE TO NITROGEN FERTILIZATION

2nd internode (a)

Constituents	Applications	25.6.54	6.7.54	14.7.54	22.7.54
Acid extractable carboyhdrates	N_0	8.77	11.21	8.78	9.00
	N	9.18	12.41	10.55	9.59
Total Phosphorus	N_0	0.111	0.117	0.125	0.127
	N	0.193	0.206	0.150	0.142
Acetic Acid solu.phosphorus	N_0	0.086	0.091	0.075	0.073
	N	0.090	0.097	0.067	0.062
K	N_0	2.77	2.69	1.68	1.46
	N	2.66	2.57	1.73	1.61
Ca	N_0	0.289	0.267	0.363	0.405
	N	0.276	0.285	0.393	0.374
Mg	N_0	0.197	0.305	0.053	0.053
	N	0.199	0.196	0.066	0.058

3rd internode (b)

Acid extractable carbohydrate	N_0	8.65	12.59	11.28	10.64
	N	10.15	15.94	13.16	12.43
Total phosphorus	N_0	0.1219	0.1478	0.1389	0.1335
	N	0.1218	0.1166	0.1326	0.1296
Acetic acid solu.phosphorus	N_0	0.072	0.0838	0.0869	0.0821
	N	0.0743	0.0874	0.0795	0.0670
K	N_0	2.63	2.46	1.49	1.29
	N	2.56	2.18	1.40	1.32
Ca	N_0	0.310	0.223	0.323	0.355
	N	0.309	0.260	0.334	0.358
Mg	N_0	0.159	0.248	0.031	0.039
	N	0.144	0.150	0.058	0.040

mean for four values

TABLE IX (c)

Corms

Constituents	Applications	25.6.54	6.7.54	14.7.54	22.7.54
Acid extract-able carbohydrates	N_O	52.47	55.22	57.65	43.50
	N	44.09	55.33	70.28	47.31
Total phosphorus	N_O	0.116	0.131	0.164	0.162
	N	0.125	0.151	0.187	0.186
Acetic acid sol.phosphorus	N_O	0.092	0.123	0.118	0.115
	N	0.129	0.141	0.106	0.075
K	N_O	2.50	2.38	1.16	1.16
	N	2.34	1.64	1.39	1.20
Ca	N_O	0.164	0.215	0.282	0.312
	N	0.152	0.121	0.222	0.237
Mg	N_O	0.135	0.177	0.024	0.033
	N	0.128	0.115	0.028	0.035

TABLE X

MEAN# VALUES (AS PER CENT OF OVEN D.M.) FOR THE DIFFERENT CONSTITUENTS OF THE CORM SAMPLES AS A RESULT OF PHOSPHORUS APPLICATION TO THE SOIL

Constituents	Dates	NoPo	NPo	NoP	NP
Acid extractable carbohydrate	10.9.54	58.94	70.94	56.57	63.13
	7.10.54	58.75	64.88	58.44	63.19
Total N	10.9.54	0.218	0.255	0.196	0.190
	7.10.54	0.3472	0.3752	0.3332	0.329
Acetic acid soluble P.	10.9.54	0.1011	0.1086	0.1184	0.1141
	7.10.54	0.1141	0.1156	0.1289	0.1214
Total P	10.9.54	0.1647	0.1657	0.1764	0.1558
	7.10.54	0.1955	0.1982	0.2040	0.1881
Total Mg	10.9.54	0.03	0.0415	0.0365	0.028
	7.10.54	0.03	0.0415	0.0365	0.028
Total K	10.9.54	1.081	1.114	1.129	1.121
	7.10.54	1.081	1.104	1.138	1.110
Total Ca	10.9.54	0.315	0.345	0.294	0.306
	7.10.54	0.368	0.432	0.332	0.417
Moisture	10.9.54	61.89	62.31	63.52	62.48
	7.10.54	62.55	61.79	62.29	61.49

Mean for four values

APPENDIX D

VALUES FOR NITROGEN (AS % OF OVEN D.M.)

SET UP FOR ANALYSIS OF VARIANCE

(a) 2nd internode
with four replicates

Dates	25.6.54		6.7.54		14.7.54		22.7.54			Reps.
Appln.	No	N	No	N	No	N	No	N	Sums	Rep.
	1.075		0.812		0.644		0.610		T_I	I
		1.189		0.875		0.689		0.717	T_{II}	
	0.938		0.756		0.650		0.504		T_{III}	Rep.
		1.148		0.857		0.700		0.706	T_{IV}	II
	1.098		0.851		0.650		0.605		T_V	Rep.
		1.271		0.829		0.666		0.689	T_{VI}	III
	0.946		0.627		0.571		0.515		T_{VII}	Rep.
		1.187		0.868		0.672		0.717	T_{VIII}	IV
	D_1		D_{II}		D_{III}		D_{IV}		SUM.	

(b) 3rd internode
with four replications

Dates	25.6.54		6.7.54		14.7.54		22.7.54			Reps.
Appln.	No	N	No	N	No.	N	No	N	Sums	
	0.706	0.68	0.532	0.567	0.493	0.409	0.347	0.515	T_I T_{II}	Rep.I
	0.717	0.683	0.567	0.549	0.498	0.437	0.263	0.398	T_{III} T_{IV}	Rep.II
	0.706	0.795	0.526	0.543	0.493	0.403	0.342	0.403	T_V T_{VI}	Rep.III
	0.689	0.655	0.543	0.605	0.470	0.454	0.347	0.414	T_{VII} T_{VIII}	Rep.IV
	D_1		D_{11}		D_{111}		D_{IV}		Sum	

(c) Corms

Dates	25.6.54		6.7.54		14.7.54		22.7.54			Reps.
Appln.	No	N	No	N	No	N	No	N	Sums	
	0.151	0.179	0.147	0.147	0.146	0.196	0.129	0.291	T_I T_{II}	Rep.I
	0.112	0.157	0.112	0.140	0.146	0.196	0.157	0.264	T_{III} T_{IV}	Rep.II
	0.157	0.190	0.140	0.134	0.146	0.190	0.140	0.289	T_V T_{VI}	Rep.III
	0.151	0.168	0.123	0.179	0.123	0.196	0.129	0.247	T_{VII} T_{VIII}	Rep.IV
	D_I		D_{II}		D_{III}		D_{IV}		SUM	

RESULTS OF ANALYSIS OF VARIANCE ON

TOTAL NITROGEN

(a) Stem leaf, 2nd internode
(analysis on 4 replications)

Source	df.	S.S.	M.S.	F.	Significant .05	.01
Main plot	7	1767.89				
Replications	3	285.09	95.03	0.91	9.28	---
Nitrogen (N)	1	1170.07	1170.07	11.23	"	---
Error (a)	3	312.73	104.24			
Dates of supply	3	11382.77	3794.26	427.29	3.16	5.09
Dates XN	3	207.69	69.23	7.80	"	"
Error (b)	18	159.69	8.88			
Grand Total	31					

(N.B. All figures multiplied by 100)

(b) <u>Stem leaf 3rd internode</u>
(analysis on 4 replications)

Source	df.	S.S.	M.S.	F	Significance at .05	.01
Main plot	7	4049	....			
Replications	3	1315	438.0	3.395	9.28	
Nitrogen (N)	1	2346	2346.0	18.00	"	
Error (a)	3	388	129.0			
Dates of supply	3	471961	157320	94.15	3.16	5.09
Dates XN	3	29902	9967	5.96	"	"
Error (b)	18	30090	1671			
Grand Total	31	536002	....			

(All figures multiplied by 1000)

(c) Corms
(only two replications)

Source	df	S.S.	M.S.	F.	Significance at .05	.01
Main plot	3	137.18	45.73			
Replications	1	9.00	9.00	18.37	161	4,052
Nitrogen (N)	1	127.69	127.69	260.59	"	"
Error (a)	1	0.49	0.49			
Dates of sampling	3	109.57	36.53	10.47	4.76	9.78
Dates XN	3	70.09	23.36	6.69	"	"
Error (b)	6	20.95	3.49			
Grand total	15					

(N.B. All figures multiplied by 100)

APPENDIX E

ACTIVITY COUNTS OF THE TOP THREE INCHES OF SOIL

Background counts = 54　　　　Samples collected on 5.9.54

	Counts per minute			
Plot I	1st inch	2nd inch	3rd inch	4th inch
	81, 89 101	94, 93 95	67, 84 87	54
Plot II	90, 95 91	104, 99 100	93, 72 67	57, 54 54
Plot III	160, 74 135	56, 70 82	75, 77	-
Plot IV	78, 76 78	59, 100 59	76, 62 63	-
Plot V	108, 100 98	88, 83 74	65, 70 73	-
Plot VI	72, 80 81	64, 67 69	68, 59 69	-
Plot VII	70,73 68	55, 59 67	60, 62 63	-
Plot VIII	129, 100 79	84, 83 85	82, 90 59	-