THE APPLICATION OF RAPID CHEMICAL TESTS TO THE DIAGNOSIS OF MINERAL DEFICIENCIES IN HORTICULTURAL CROPS

I. CROPS GROWN ON A MANURIAL TRIAL

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SYNOPSIS

A chemical tissue test method, designed to determine the mineral status of field crops, which has been tested to diagnose mineral deficiencies in potato and cauliflower, is described. The procedure comprises a specialized method of sampling fresh plant material, followed by the extraction of the easily soluble nutrients from selected portions of the plants and their subsequent estimation by colorimetric and turbidimetric tests. A buffered solution consisting of glacial acetic acid and sodium acetate (100 gm. sodium acetate and 30 ml. of glacial acetic acid in 1 litre of distilled water, at pH 4·8) is used for the extraction of the nutrients from weighed amounts of fresh tissue for a standard time, viz. 15 minutes. The tissue extract is tested for potassium, magnesium, calcium, phosphorus, nitrate nitrogen and manganese. The detection of the last at deficiency levels involves the use of specially purified reagents. Nutrient standards for the various elements have been fixed for a number of plants and threshold deficiency values are given which coincide with the development of visual symptoms of the respective deficiencies. It is shown that the tissue test data reflect manurial and cultural treatments and are in accord with results of the visual method and those of ash analysis of the plants. The method is recommended as complementary to visual diagnosis for determining deficiencies in field crops and as a quick procedure for assessing the mineral status of plants. It replaces the time-consuming process of ash analysis.

THE practice of determining the mineral requirements of crop plants by means of chemical tissue tests originated in America. Early work was done by Hoffer (22) who made qualitative tests for Fe and K in fresh maize stalks (*Zea mays*) and showed that Fe accumulated at the nodes. He noted an inverse relation between K and Fe status of the nodal tissues and used this staining method to confirm K deficiency. McGillivray *et al.* (25), using thin cross-sections of leaf petioles of tomatoes immersed in platinum 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 K status of the plants.

A later development was the study by Gilbert and Hardin (18) of the nutrient content of expressed sap in regard to N, P and K, by grinding fresh material and straining through a muslin mesh. McCool and Weldon (24) used a similar method for K and P using sap extracted at a pressure of ½ to 1 ton per sq. in. Fonder (14, 15) and Cook (6, 7) examined the sap of beans and cereals respectively for Ca and Mg and nitrate N. Pettinger (40) showed that the sap of maize reflected the N, P, K treatments given and in some instances Cl was also included. Neller (31) determined P in plant sap of sorghum and buckwheat, and Poehlman (43) nitrate N, P, K; both showed that it was impossible to fix critical levels for the nutrients as had been recommended by Carolus working with potatoes (3, 4). Discrepancies in the composition of extracted sap have been noted by Knudson and Ginsburg (23) and Gassner and

Goeze (16, 17), especially when the pressure used to obtain it is varied or when there is a time difference in attaining the same trial pressure. Phillis and Mason (41) confirmed these variations using fresh plant tissue and it was suggested that freezing of tissues or ether treatment might be necessary to destroy the semi-permeable membranes of the cells. Work along these lines has not yielded consistent results and no publications have appeared in recent years.

The extraction of soluble nutrients has been attempted by a number of workers, using a variety of solvents. The simplest procedure is the extraction with water as used by Nightingale (36, 37) for the detection of Ca deficiency in sugar cane and pineapple, and by Marsh (27) using tobacco and maize. Page and Burkhart (38) have used boiling water for the extraction of nutrients from fresh material for diagnosis of NPK and deficiencies in peanut and cotton. The use of an organic acid, 2 per cent. acetic acid, for extraction, was introduced by Emmert (8, 9, 10, 11, 12, 13) for detecting nitrate N, P and K, and later Carolus (3, 4) extended it to include Ca and Mg in vegetable crops. These investigators as well as Hill (21), Cook (6, 7) and Ulrich (47) have reported satisfactory results but New Zealand work reported by McNaught (26) does not substantiate these claims.

The extraction of nutrients by the Purdue method devised by Thornton *et al.* (45, 46) is made by the chemical reagents used for detecting the required nutrient, e.g. 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 out into solution. The results are usually reported in four categories, viz. very high, high, medium and low, but numerical data for these categories have not been reported. This method has been used by Scarseth (44) Atkinson (2) and Wark (51) for the diagnosis of mineral deficiencies in crop plants.

Carolus (3, 4), Hester (20) and Peech and English (39) have used a Waring blendor for extracting the nutrients from fresh tissues. Carolus used a 2 per cent. acetic acid but Hester adopted the acetate buffer solution (pH 4·8) as developed by Morgan (28, 29, 30) for the latter's soil testing system. Hester has suggested values for nitrate N, P and K which correspond to maximum yield data in vegetable crops. These methods, however, have the disadvantage of not being usable in the field.

A tissue test system has been developed at Long Ashton (33, 34, 35, 42) which is comparable to Thornton's method for speed. It includes a more precise quantitative determination of the soluble nutrients and is capable of use in the field.

This paper describes the application of the method in the study of the mineral status of potato (vars. Majestic and Kerr's Pink) and cauliflower grown on a long term manurial trial. It is shown that the correlation between the tissue test data and results of other methods used to determine mineral status, in particular those of the visual method (49) and ash analysis of the foliage, is usually close.

EXPERIMENTAL.

Materials.—Table I contains data concerning the site, soil characters, crops grown, and nutritional problems occurring at the centre, as well as the sampling times for tissue tests and ash analysis.

At this centre use was made of a long term manurial trial in which six treatments are represented: Nil, farmyard manure, complete fertilizer (NPK), Omit nitrogen

TABLE I.

Centre.	Sampling dates for tests and ash analysis.	Soil characters.	Crop and variety.	Nutritional problems.
A. Long Ashton Plot 17A	2/7/45 12/6/46 25/7/45 8/7/46 23/8/45 20/8/46	Calcareous sandy loam derived from red drift overlying Keuper Marl.	Potato, Majestic and Kerr's Pink	N, P (trace), K and Mg deficiencies.
	17/7/45 29/7/46 27/8/45 29/8/46 21/9/45 25/9/46		Cauliflower, Majestic	N, K and Mg deficiencies

(PK), Omit phosphorus (NK), Omit potassium (NP). The annual rates of application per acre were as follows :

F.Y.M.	10 tons per acre.
Nitrogen	4 cwt. of nitrochalk per acre.
Phosphorus	3 cwt. of superphosphate per acre.
Potassium	2 cwt. of sulphate of potash per acre.

The treatments occur in triplicate and magnesium sulphate at 1 cwt. per acre is applied annually to one set of the six treatments (Block II).

The surface soil and subsoil contain approximately 5 per cent. and 1 per cent. free lime respectively with a pH of about 7·2 to 7·4 in the top soil.

METHODS FOR CHEMICAL TISSUE TESTS AND ASH ANALYSIS.

Selection of plant parts.

Details of the field sampling method have been given in previous papers (33, 34, 35, 42). The importance of taking morphologically similar parts of plants for comparison has been stressed in view of the presence of nutrient gradients within plants. The leaves taken for examination are as follows :

Potato and tomato : Leaves from the mid-stem portion of plants.

Cauliflower : The first upright leaf outside incurled leaves.

Apple and black currant : The 3rd and 4th basal leaves taken from well exposed leader shoots.

Preparation of test samples.

(*a*) *Tissue tests.* Small petiole portions taken from numerous leaves at approximately half-way between leaf attachment and base of lamina are used. In the cauliflower, mid-rib portions of comparable girth near the tip of the lamina are usually taken.

(*b*) *Ash analyses.* The leaves, including the petiole, are dried for 48 hours in a specially aerated oven and are then milled. When a wet digestion procedure is used for the removal of organic matter, approximately 5 gm. of dry matter is placed in a 40 ml. pyrex beaker fitted with a watch glass and 10 ml. of HNO_3 (conc.) added per 1 gm. This is heated on an Argand burner for approximately 35 minutes to remove ammonia. After cooling, 1 ml. of $HClO_4$ and 0·1 ml. H_2SO_4 per gm. of dry matter are added slowly and heating is continued until the " frothing " stage, when the oxidation of organic matter takes place. At this stage the oxidation process is continued at a lower temperature until the silica is dehydrated. Fifty ml. of H_2O is

then added and further heated for 15 minutes, cooled and filtered through a 42 Whatman paper into a graduated flask.

Extraction of soluble nutrients

Morgan's reagent, consisting of 100 gm. of sodium acetate and 30 ml. of glacial cetic acid (pH 4·8) is used for the extraction of soluble K, Mg, Ca, P, nitrate N, l and Mn (for the last when present in excess). The method recommended in revious papers (33, 34) is used, viz. 4 gm. of finely chopped tissue is immersed in ml. of Morgan's reagent for 15 minutes and the resultant solution is then poured off quickly into a clean specimen tube, using a glass funnel fitted with a small plug of cotton wool.

The tissue extract is usually clear, but those of fruit tree petioles may be coloured by flavones, tannins and other phenolic compounds. These may be removed before testing by using 0·2 gm. of specially purified carbon (Darco G.60 grade) per 25 ml. of extract and stirring with a glass rod for 30 seconds, then filtering through a No. 41 Whatman paper into another specimen tube. A purified carbon may be prepared by boiling the B.D.H. product with 5 N. HCl and rinsing with hot distilled water. The addition of sodium chloride facilitates the removal of phosphate from the carbon. Rinsing with hot water should continue until Cl cannot be detected in the leachate and then, after drying, the carbon is tested for mineral elements by shaking with Morgan's solution. The results obtained on blank determinants are usually <1 p.p.m. PO_4; 0·2 p.p.m. Mg; Nil K; Nil Ca.

For the detection of deficiency levels of Mn in crop plants, a purified Morgan's reagent is used (34). The last traces of Mn may be removed from sodium acetate by using a modification of Arnon and Stout's adsorption method (1) as follows: 100 gm. of sodium acetate are dissolved in 500 ml. of distilled water and autoclaved at a temperature of 120° C. for three-quarters of an hour in the presence of 20 gm. of $CaCO_3$ and 20 ml. 20 per cent. solution of Na_2HPO_4 and $CaCl_2$ respectively. The presence of 5 gm. each of Na_2CO_3 and $NaHCO_3$ helps to remove Mn completely. It has also been noted that should the Mn impurity be $<40 \times 10^{-3}$ p.p.m. Mn, the addition of 5 ml. of 25×10^{-3} p.p.m. to the autoclaved mixture assists in the complete removal of Mn from solution, i.e. $<1 \times 10^{-3}$ p.p.m. Mn.

Chemical tests used.

Since the initial progress report (32, 42) several modifications and refinements have been made. The chemical tests used are given in Table II.

The following notes are added to supplement the details in the table.

Potassium. The sodium cobaltinitrite should be freshly prepared every three weeks, thoroughly aerated before use and stored in the refrigerator. After 2 ml. of isopropyl alcohol is added, the tube is shaken vigorously, using a clockwise movement for one minute to effect a complete precipitation of the potassium sodium cobaltinitrite. The shaking procedure must be standardized for consistent results. The turbidity is determined immediately, using the Spekker Absorptiometer with red Ilford filters and a water setting of 1, using 1 cm. cells.

Calcium. After the addition of the oxalate the tube is shaken as for the K method for 2 minutes and is then allowed to stand for 15 minutes. The turbidity is determined on the Spekker, using blue filters with a water setting of 0.8, and 1 cm. cells. Care must be taken to transfer the total precipitate to the Spekker cells.

Table III.

Relation between tissue test values and actual concentration of nutrients in parts per million in the Morgan's Extract (normal and deficiency levels).

	Potassium.		Calcium.			Magnesium.		Manganese.	Phosphate.		Nitrate.	
	Cauliflower, Tomato, Black currant.	Potato, Apple.	Tomato, Potato.	Apple.	Cauli-flower.	Apple.	Potato, Tomato, Cauliflower, Black currant.	Tetramethyl method. Potato, Cauliflower.	Potato, Tomato, Black currant, Apple.	Cauli-flower.	Potato.	Tomato, Cauli-flower.
High + ..			270				14	80×10^{-3}				350
High ..	200	150	200	175	300	8	12	70×10^{-3}	30	60	150	300
High − ..	165	125	165	150	250	7	10	60×10^{-3}	25	50	125	250
Medium +	130	100	130	125	200	6	8	50×10^{-3}	20	40	100	200
Medium ..	95	75	95	100	150	5	6	40×10^{-3}	15	30	75	150
Medium −	60	50	60	75	100	4	4	30×10^{-3}	10	20	50	100
Low + ..	25	25	25	50	50	3	2	20×10^{-3}	5	10	25	50
Low ..	10	10	10	10	25	2	1	10×10^{-3}	<5	<10	<10	<25
Low − ..	<10	<10	<10	<10	<25	1	<0·5	5×10^{-3}				

Magnesium. For most of the Long Ashton work before the war, Titan Yellow (Dr. Grubler grade) has been used and this has proved most reliable. Titan Yellow however, may vary in strength and quality with batches and thus it is necessary to recrystallize and standardize the product. The latter may be conveniently done by

TABLE II.

Chemical tests.

(Using Morgan's solution as the extracting solution.)

Kations or anions tested.	Reagents used.	Coloration or turbidity ranges.	
		Minimum.	Maximum.
Potassium (K) ..	0·2 ml. 35% sodium cobaltinitrite. 1 ml. 50% glycerin. 2 ml. isopropyl alcohol.	Clear reddish brown solution	Deep canary yellow turbidity.
Calcium (Ca) ..	2 ml. 50% glycerin. 5 ml. saturated solution of ammonium oxalate.	Colourless solution.	Greyish white turbidity.
Magnesium (Mg)	0·2 ml. 0·15% Titan yellow.* 0·5 ml. 2% hydroxylamine hydrochloride. 0·5 ml. 5% sucrose. 2 ml. 10% sodium hydroxide.	Straw yellow solution.	Salmon pink colour.
Manganese (Mn) (present in excess)	0·5 ml. 10% trioxymethylene sulphate. 2 ml. 10% sodium hydroxide.	Colourless solution.	Deep cherry colour.
Manganese (Mn) (sensitive test for deficiency levels)	2 ml. potassium periodate.* (Saturated solution.) 0·4 ml. 1% tetramethyl.*	Pale blue colour.	Deep blue colour.
Nitrate (NO_3) ..	2 ml. 25% W/V phenoldisulphonic acid in sulphuric acid. 5 ml. H_2O. 10 ml. 30% ammonia until alkaline.	Colourless.	Deep buff colour.
Phosphate (PO_4)	2 ml. 4% ammonium molybdate. 1 ml. 1% hydroquinone. 2 ml. { 26% potassium carbonate. 5% sodium sulphite.	Faint blue colour.	Deep Mediterranean blue colour.
Chloride (Cl) ..	2 ml. N/50 silver nitrate. 3 drops HNO_3 (conc.).	Colourless solution.	White turbidity.

* Should be recrystallized and standardized before use.

making up the recrystallized material in alcohol (see Table II) and then adding 0·1, 0·2, ... 1·0 ml. respectively to a series of tubes containing 5 ml. of 5 p.p.m. Mg standards and completing the test. Matching is then done with a Lovibond disc and the volume of Titan Yellow required for perfect match is always used for that batch of the dye. Titan Yellow standards must be checked at frequent intervals.

Manganese (*at deficiency levels*). Tetramethyldiaminodiphenylmethane should be recrystallized before use, using redistilled methyl alcohol. The tetrabase should be colourless when dissolved in acetone. The colour is more stable when the tissue extracts are cooled in ice.

Nitrate. Nitrates are usually determined by evaporating the solution to dryness and then adding phenoldisulphonic acid or brucine in H_2SO_4. Attempts were made initially to add phenoldisulphonic acid to macerated tissues but charring masked the yellow colour. It was found that the addition of phenoldisulphonic acid to the tissue extract, followed by ammonia after cooling, gave differences during the early growth period between normal plants and those deficient in nitrogen. Later in the season it was found that nitrate N fell to low values in tissue extracts, irrespective of treatment, and the evaporation of the tissue extract and addition of phenoldisulphonic acid to the residue did not produce a nitrate colour, thus proving the absence of nitrate N in plants late in the season.

Nitrates could not be detected in either apple petiole extracts or those of legumes even though the extracts were evaporated to dryness and phenoldisulphonic acid added to the residue.

Results.

Nutrient standards.

The assessment of colour and turbidity is made by using a Lovibond Comparator and discs, or, for more precise work, by prepared standards made up in Morgan's solution calibrated by means of the Spekker Absorptiometer. An empirical scale of values is used for each nutrient comprising High (H), Medium (M), Low (L), and Trace (Tr), and these categories may be further divided by the use of a + or − sign. The values are fixed for each crop plant. Approximate relationships between tissue test categories and concentration of nutrients in the extracts of various crop plants are given in Tables III and IV.

Table IV.

Relation between visual tissue test groupings and concentration of Mn and Cl in parts per million in the Morgan's Extract (*normal and toxic levels.*)

Visual oupings.	High ++++	High +++	High ++	High +	High	High −	Trace ++ or medium.	Trace +	Trace
Mn	10	8	5	3	2	1	0·8	0·5	0·2
Cl	150	125	100	75	50	25	15	10	5

A series of colour standards using bromothymol blue, thymol blue and bromophenol blue are used in the determination of Mn by the tetrabase method. Matching of Mn in the tissue extract should be made within 1 minute of adding the reagents and not later than 2 minutes, as the reaction is catalytic and, at high Mn levels, the blue colour gives way to fluorescent red and green, making matching with colour standards difficult. Data relating to the making up of the colour standards are

found in Table V. These standards were matched individually with Mn standards made up in the Morgan's reagent.

TABLE V.

Colour standards for the determination of manganese in tissue extracts (tetramethyl test) showing amounts of bromothymol blue, thymol blue, bromophenol blue and ammonia (volume in mls.).

	Lettered labels to facilitate quick matching.									
Indicator used.	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.
Bromothymol blue ..	0·03	0·06	0·09	0·125	0·15	0·20	0·25	0·30	0·40	0·50
Thymol blue ..				0·025	0·05	0·10	0·10	0·15	0·15	0·20
Bromophenol blue ..							0·05	0·10	0·15	0·20
2% NH_4OH ..	13	13	13	13	13	15	18	18	18	20
Mn in p.p.m. $\times 10^{-3}$ ..	2–3	5	10	15	20	30	40	50	60	80

Tissue extracts must be chilled in an ice salt mixture for 5 minutes before testing.

At this centre, tissue tests for K, Mg, Ca, PO_4, nitrate N, and Mn were made at six fortnightly intervals throughout the 1945 and 1946 seasons using two varieties of potato (Majestic and Kerr's Pink) and cauliflower. Ash analyses of the Majestic variety and of the cauliflower were made three times during the seasons 1945 and 1946, coinciding with tissue test determinations. The data are presented as histograms.*

The results for both crops are considered under the following heads.

TISSUE TESTS IN RELATION TO MANURIAL AND CULTURAL TREATMENTS.

A series of histograms representing the mean of the six seasonal tissue test values for K, Mg, Ca, PO_4, nitrate N and Mn in potato (Majestic and Kerr's Pink) and in cauliflower for the seasons 1945 and 1946 are given in Figs. 1 to 5.

Potassium. There is a significant difference between treated and non-treated K plants and the NP (Omit K) levels are usually lower than in the Nil potato and cauliflower. The K values in potato, where K is omitted, are lower than in cauliflower on the corresponding plots, which is in agreement with the greater susceptibility of the former to K deficiency.

Magnesium. The Mg content of PK (Omit N) plants are lower than those of other treatments in Blocks I and III, and this is particularly marked in the latter. Block II plants reflect the magnesium sulphate soil treatment but the Omit N plants still show the lowest Mg value within the block. During the 1946 season the Mg level in the NPK plants (Blocks I and III) is also low, probably due to the wet season

* Data in tabular form are available for consultation at Long Ashton.

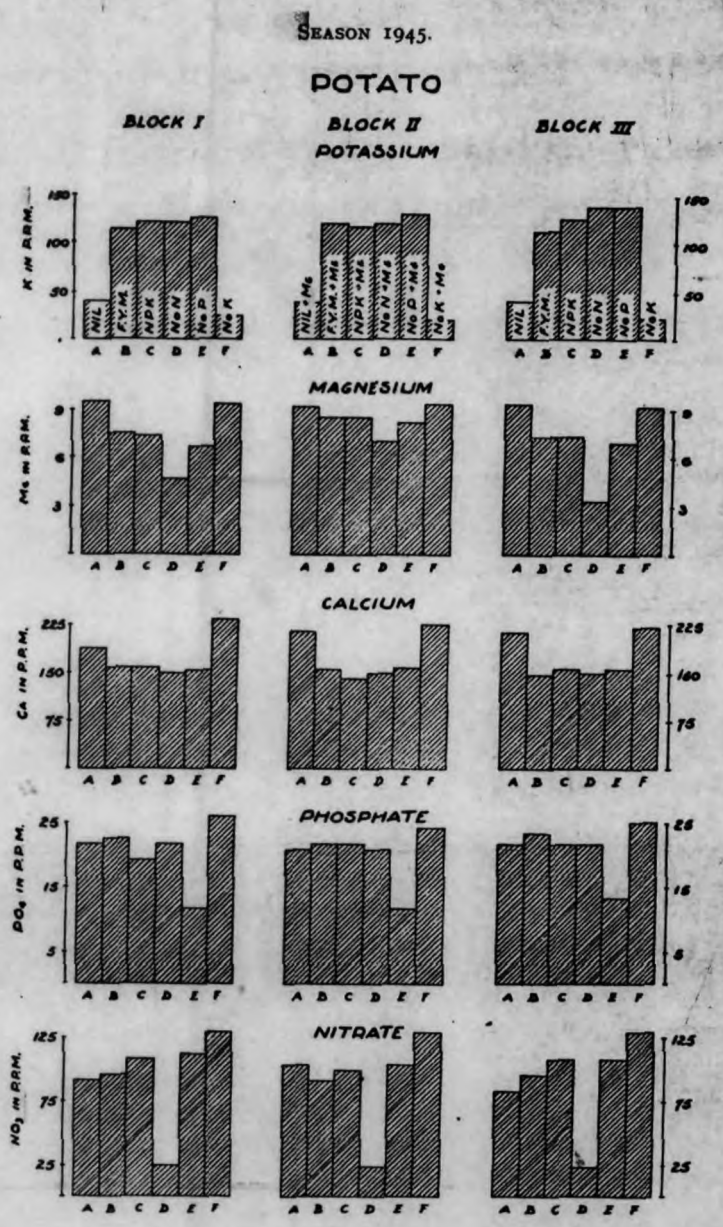

FIG. 1.

Histograms representing the mean of the six seasonal tissue test results for K, Mg, Ca, PO_4 and NO_3 in POTATO (Majestic) growing under various manurial treatments. Season 1945.

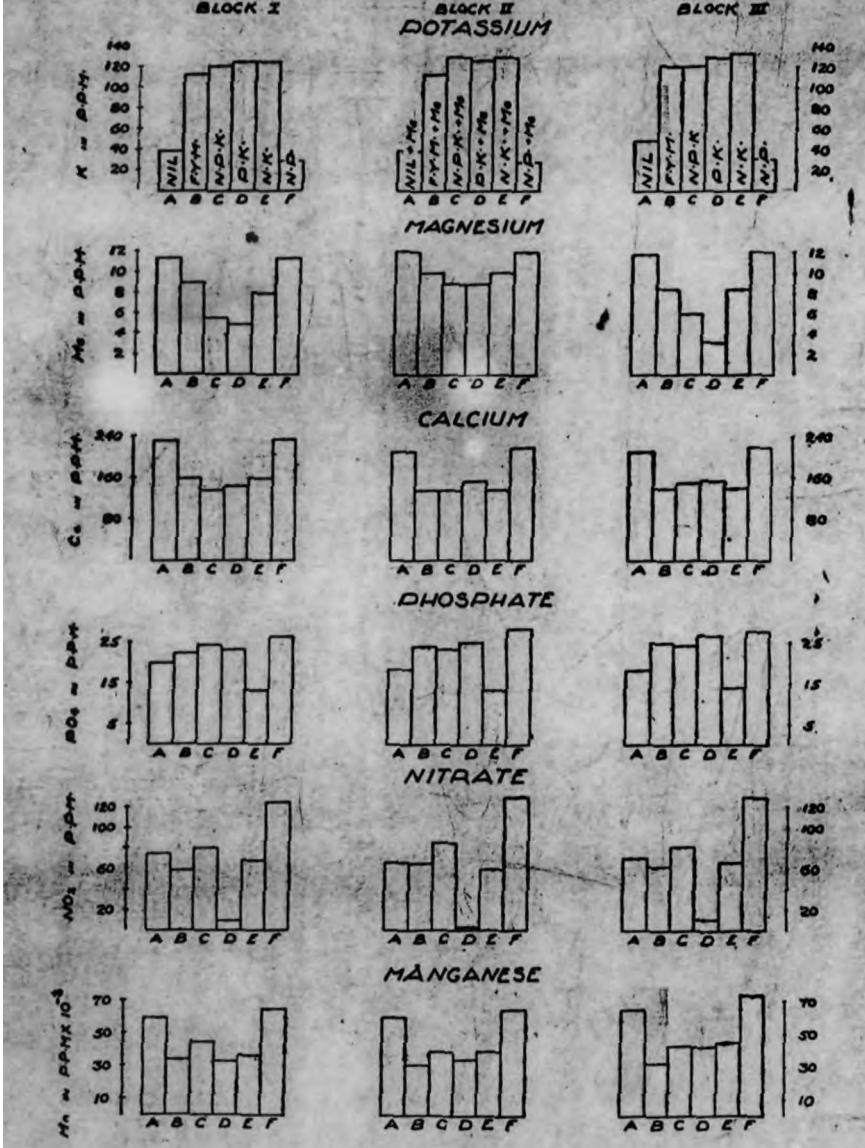

FIG. 2.

Histograms representing the mean of the six seasonal tissue test results for K, Mg, Ca, PO_4, NO_3 and Mn in POTATO (Majestic) growing under various manurial treatments. Season 1946.

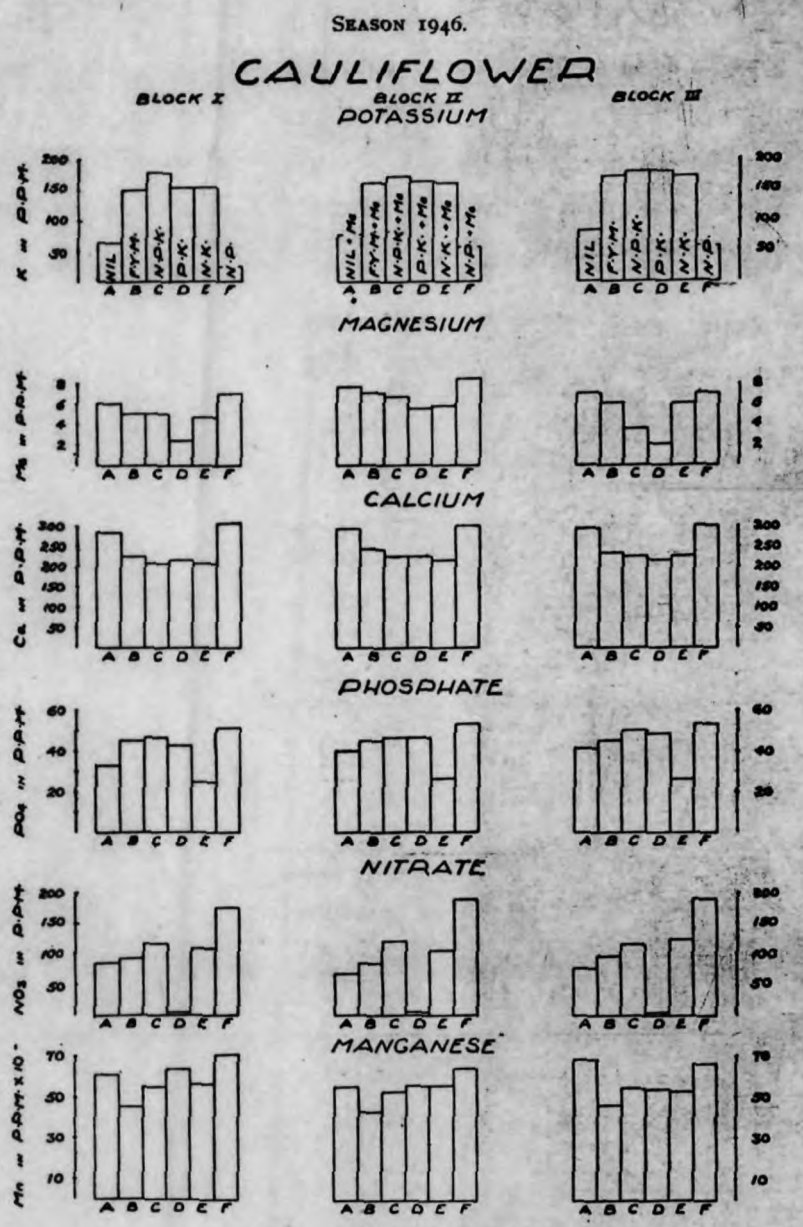

FIG. 5.

Histograms representing the mean of the six seasonal tissue test results for K, Mg, Ca, PO_4, NO_3 and Mn in CAULIFLOWER growing under various manurial treatments. Season 1946.

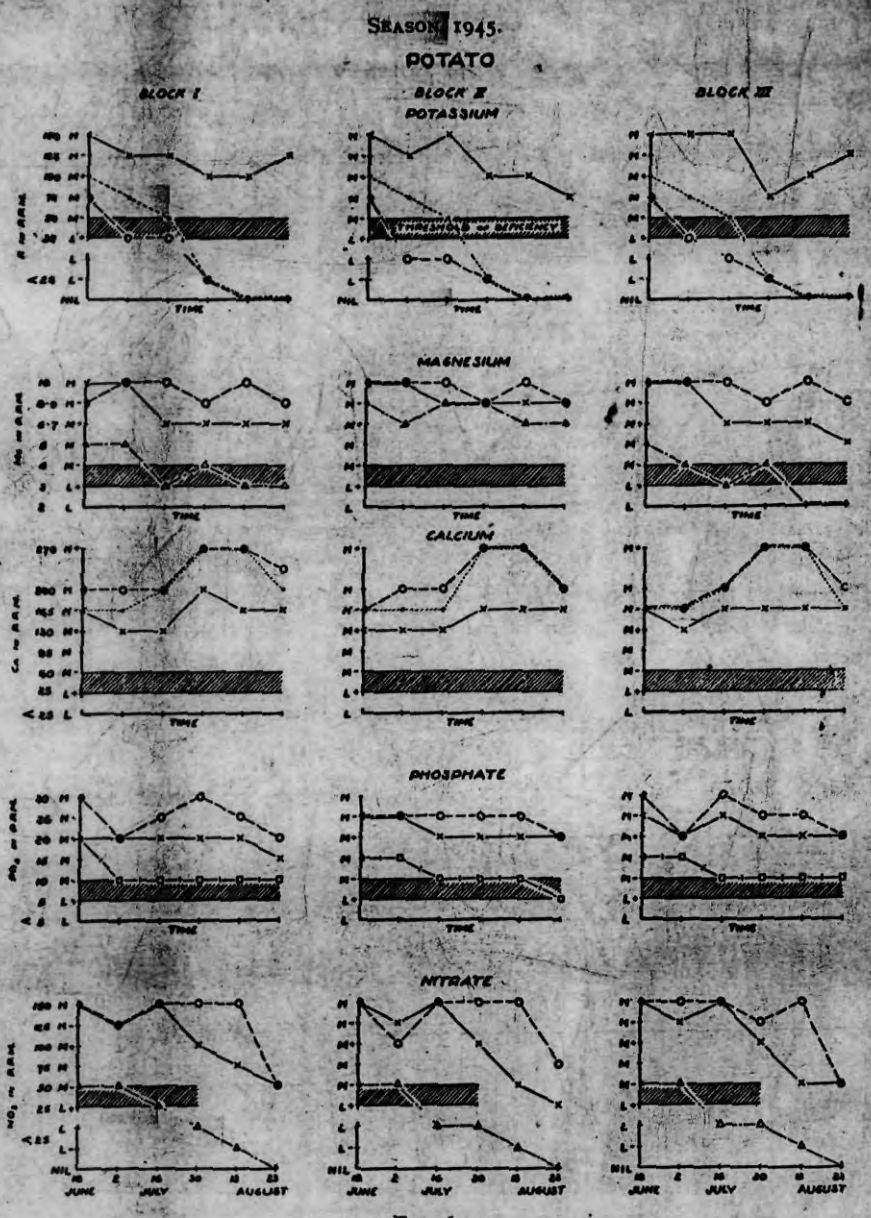

FIG. 6.

The Seasonal Cycles of soluble K, Mg, Ca, PO_4 and NO_3 in POTATO (Majestic).
The following treatments are represented :
(1) × ——— × NPK. (2) • ..... • Nil. (3) o ——— o NP.
(4) Δ —— – —— Δ PK. (5) □ —— | —— □ NK. Season 1945.
The shaded area represents the threshold range associated with visual signs of a deficiency.

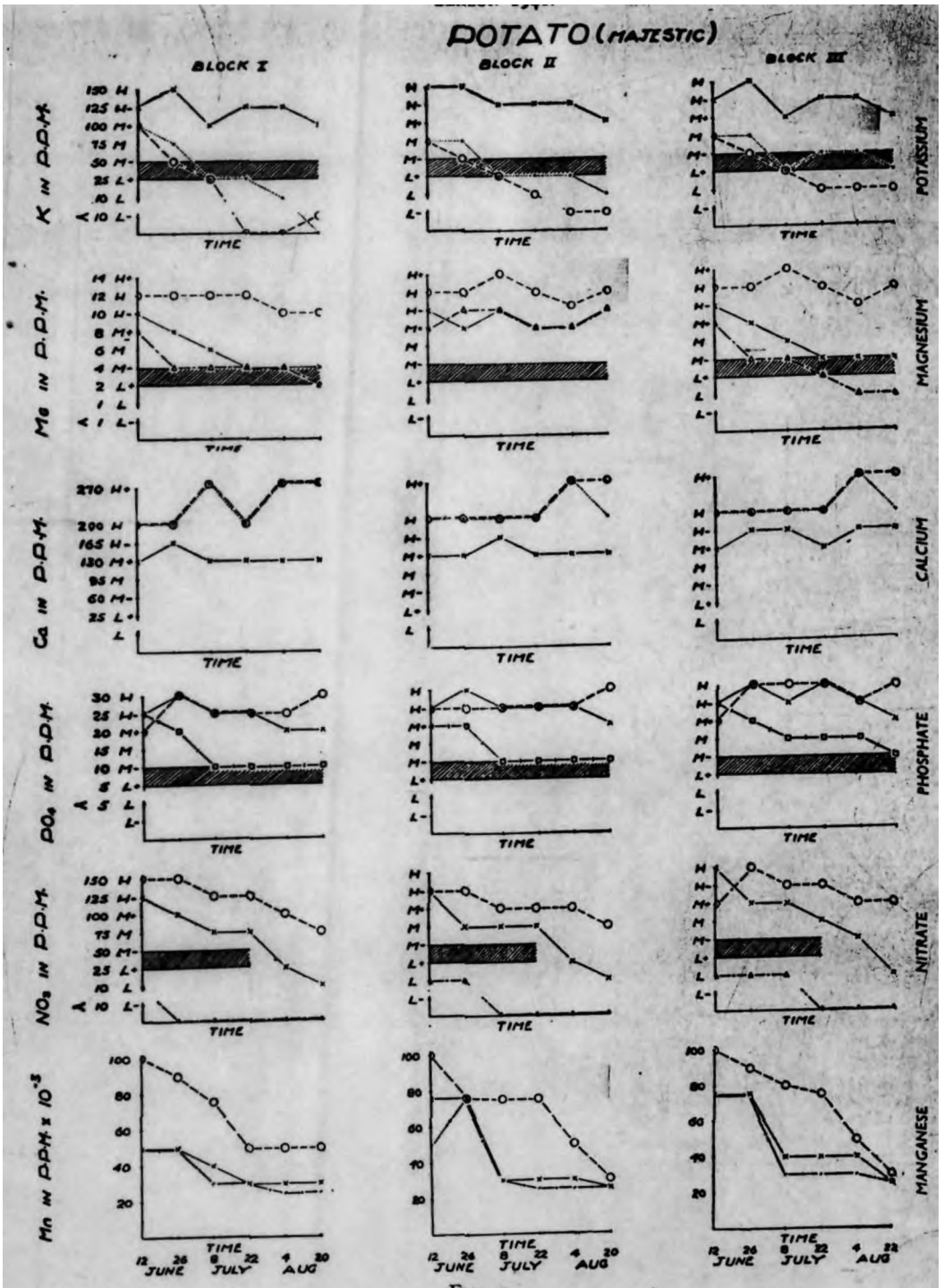

FIG. 7.

The Seasonal Cycles of soluble K, Mg, Ca, PO_4, NO_3 and Mn in POTATO (Majestic). The following treatments are represented:
(1) ×———× NPK. (2) •.....• Nil (3) ○———○ NP.
(4) △— - —△ PK. □———|———□ NK Season 1946.
The shaded area represents the threshold range associated with visual signs of a deficiency.

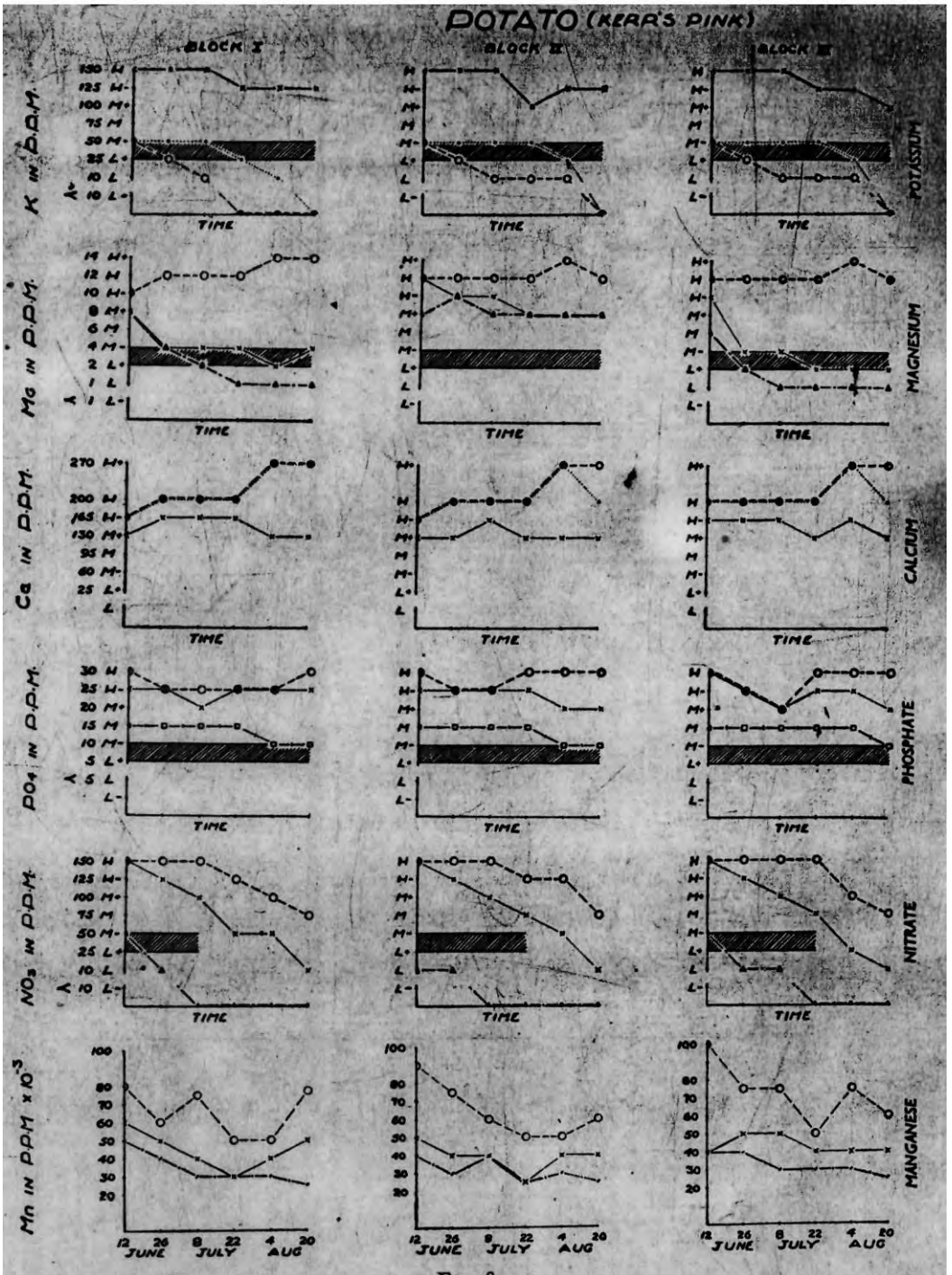

FIG. 8.

The Seasonal Cycles of soluble K, Mg, Ca, PO_4, NO_3 and Mn in POTATO (Kerr's Pink). The following treatments are represented:
(1) ×———× NPK. (2) •.....• Nil. (3) O———O NP.
(4) Δ——–——Δ PK. □——|——□ NK. Season 1946.
The shaded area represents the threshold range associated with visual signs of a deficiency.

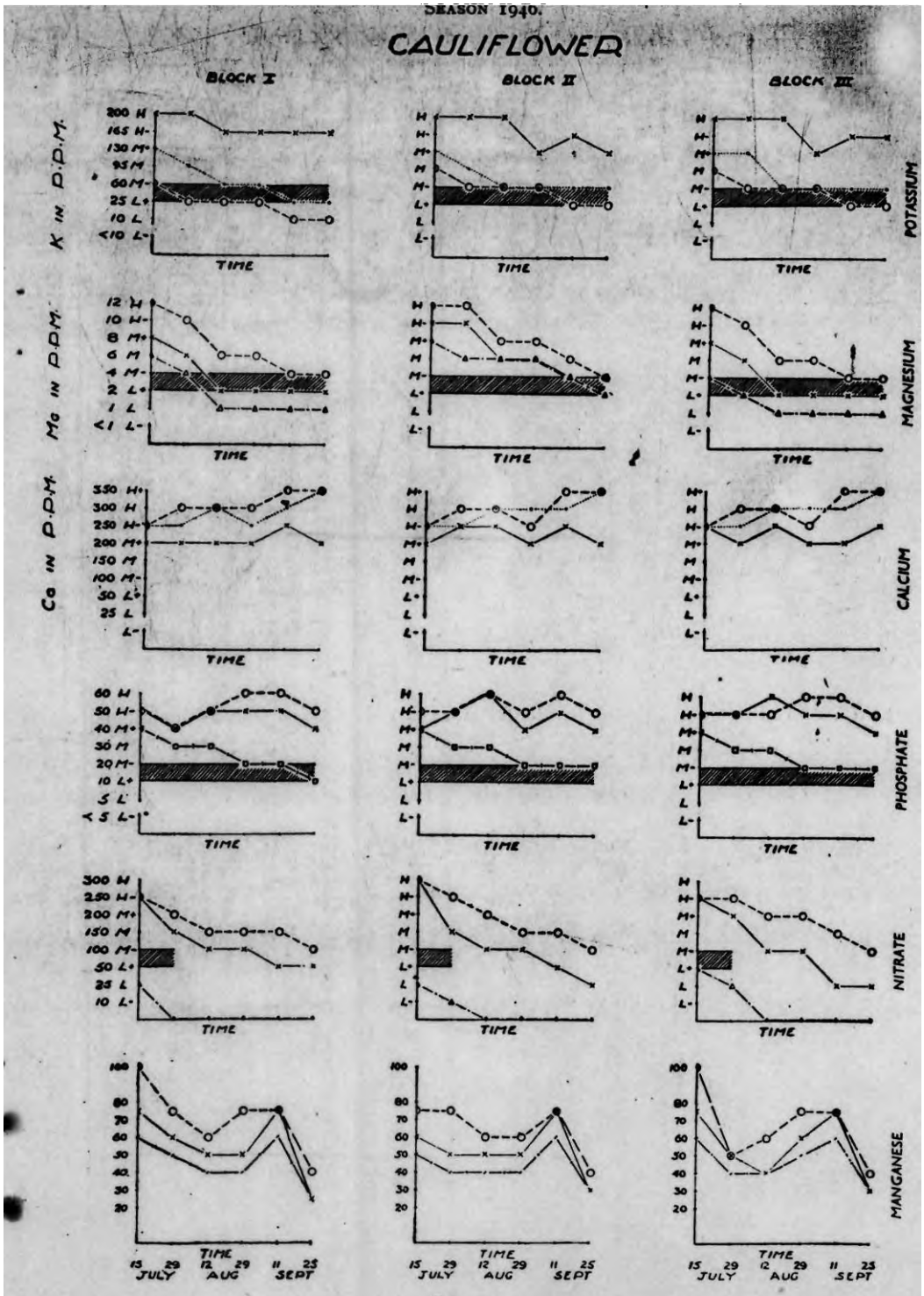

FIG. 10.

The Seasonal Cycles of soluble K, Mg, Ca, PO_4, NO_3 and Mn in CAULIFLOWER.
The following treatments are represented :
(1) ×———× NPK. (2) •.....• Nil. (3) ○———○ NP.
(4) △——-——△ PK. (5) □——|——□ Season 1946.

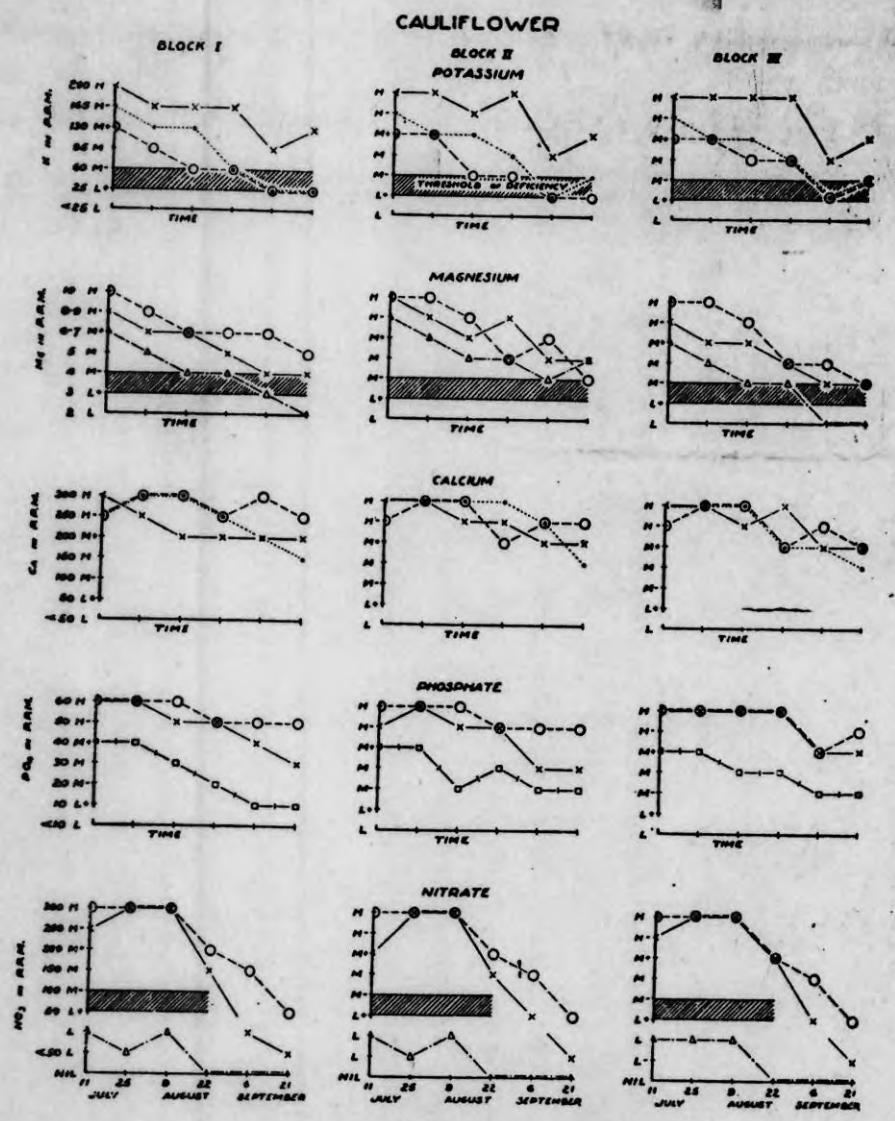

FIG. 9.

The Seasonal Cycles of soluble K, Mg, Ca, PO_4 and NO_3 in CAULIFLOWER. The following treatments are represented :

(1) ×———× NPK. (2) •.....• Nil. (3) ○———○ NP.
(4) Δ—— - ——Δ NP. (5) □——|——□ Season 1945.

The shaded area represents the threshold range associated with visual signs of a deficiency.

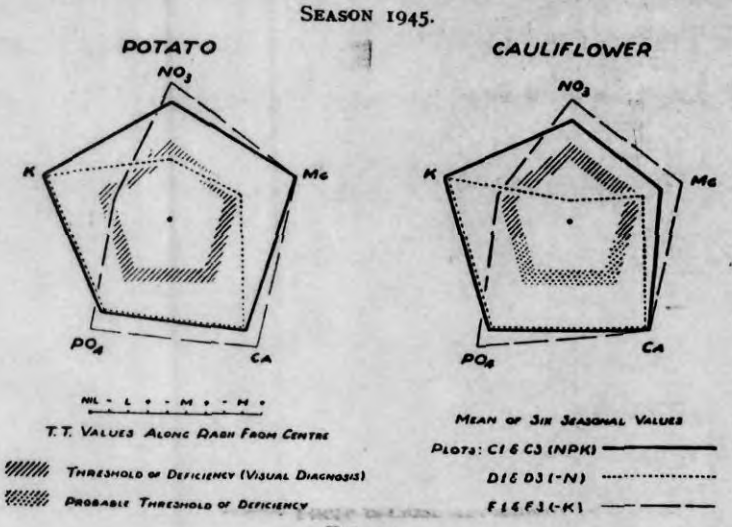

FIG. 11.

Diagrams showing the relation between soluble K, Mg, Ca, PO_4 and NO_3 in POTATO and CAULIFLOWER growing under various manurial treatments (Season 1945). The mean of the six tissue test values for each nutrient for the NPK, NP (Omit K) and PK (Omit N) treatments respectively are plotted along 5 radii of a circle (results for Blocks I and III are bulked together).

which resulted in the leaching out of nitrates from the surface soil. This was confirmed by soil analysis and by the observation that Mg deficiency was particularly severe in waterlogged patches. The Nil and NP (Omit K) plants usually have higher Mg values, thus confirming the reciprocal relation between K and Mg noted in the previous season. The fact that the Mg level in the variety Kerr's Pink is usually lower than in Majestic is in accord with the greater susceptibility of the former to Mg deficiency. The cauliflower usually has a lower Mg level than has the potato.

Calcium. The Ca levels are adequate throughout as might be expected because of the 5 per cent. free lime present in the surface soil. The values are higher when K is omitted, and this is also commonly observed when K is deficient.

Phosphorus. The Omit P treatment is reflected in the NK plants. The absence of K tends to increase the P levels in the tissues.

Nitrate nitrogen. There is a significant difference between treated and untreated N plants during both seasons. The lower values in 1946 may be due to persistent rainfall leaching nitrate N from the top soil. In the absence of K, nitrate values are higher.

Manganese. When K is omitted as in Nil and NP plants the Mn level is usually higher than in other treatments. In F.Y.M. plants, Mn values tend to be lower than in other treatments. This may be due to biological fixation of Mn which may occur in the presence of a higher level of organic matter in a calcareous soil.

TISSUE TESTS IN RELATION TO THE VISUAL SYMPTOMS AND NUTRIENT CYCLES.

In Figs. 6-10 are given the seasonal trends of the soluble nutrients in the two potato varieties and in the cauliflower for seasons 1945 and 1946. The cross hatched bands which appear in these figs. are termed " Threshold of deficiency (tissue test)

levels ". These are fixed by the level at which visual symptoms of the respective nutrient deficiencies first appear. In most instances these threshold levels coincide with the Medium minus to Low plus range but quantitative values for these levels vary with nutrient and crop plant (Table III). In some instances, however, especially late in the season, certain nutrients may fall within this range without characteristic visual signs of the deficiency being observed, e.g. nitrate N. Such low values late in the season result from the natural downward trends of the nutrients.

Shaded bands are omitted from graphs when no well-defined visual signs were observed. In a few instances the critical levels have been determined from experimental data from other centres, e.g. deficiency levels of Ca in potato were fixed from plants exhibiting " acidity " symptoms at Sutton Park (p. 107).

Tissue tests may thus be used to confirm visual symptoms but, in addition, when related to the seasonal cycles, may also indicate an impending mineral deficiecny.

Potassium. The deficiency was noted at an early growth stage in potato during both seasons and appeared first in the NP (Omit K) and later in the Nil plants. Table VI gives the relation between tissue test results and the percentage number of K deficient potato plants.

There is close agreement between the onset of visual symptoms of K deficiency and tissue test results. During seasons 1945 and 1946 symptoms were first observed in the NP treatment on June 25th and June 20th respectively in Majestic, and on June 15th, 1946, in Kerr's Pink. On August 13th and 22nd, 1945 and 1946 respectively, when 100 per cent. of the Omit K potato plants showed symptoms, no soluble potash could be detected in the tissues. In comparing data for the two seasons there is general agreement (1) that K deficiency is most severe in the NP treatments, (2) that the deficiency becomes more severe with season, and (3) that values are in accord with the severity of the symptoms noted.

The values in cauliflower did not fall as low as in potato and visual symptoms were less marked. In potato and cauliflower, medium K values in early May and July respectively were unsatisfactory as, within a week, visual signs of the deficiency appeared. Thus in these instances tissue test results may be used to anticipate the deficiency.

Magnesium. Deficiency symptoms were observed in PK (Omit N) potato in Blocks I and III in early June and July respectively in Kerr's Pink and Majestic. Tissue test values are at the threshold of deficiency at those points in the growth cycle. During 1946 the levels in NPK plants fall within the critical range whereas NP (Omit K) plants, as in the previous year, have higher values than any other treatment. Potato plants grown in Block II were free from symptoms and Mg tissue test levels are usually higher.

Data for cauliflower show that the deficiency is greater when N is omitted and K is applied. Visual signs and tissue test results agree that the deficiency is more marked than in potato, even in Mg treated and NP (Omit K) plants, values fall to within the threshold from mid to late season.

Calcium. There is a marked increase in soluble Ca when K is low. The absence of symptoms and relatively high values accord with the 5 per cent. free lime present in the surface soil.

Phosphorus. Leaf symptoms characteristic of P deficiency did not develop but the spindly growth habit has been observed in NK (Omit P) plants. The tissue test and visual data suggest that this nutrient is near the threshold of deficiency.

TABLE VI.

elation between the incidence of K deficiency and tissue test values in potato (Majestic and Kerr's Pink) at seasonal intervals in 1945 and 1946 (Block I only).

Treatment:		Nil.		NPK.		NP (Omit K).			Nil.		NPK.		NP (Omit K).	
Variety.	Date.	Percentage number of plants showing visual symptoms.	K Tissue Test.	Percentage number of plants showing visual symptoms.	K Tissue Test.	Percentage number of plants showing visual symptoms.	K Tissue Test.	Date.	Percentage number of plants showing visual symptoms.	K Tissue Test.	Percentage number of plants showing visual symptoms.	K Tissue Test.	Percentage number of plants showing visual symptoms.	K Tissue Test.
ajestic ..	18/6/45	Nil	Medium +	Nil	High	Nil	Medium	12/6/46	Nil	Medium +	Nil	High —	Nil	Medium +
err's Pink									10%	Medium —	Nil	High	25%	Medium —
ajestic ..	2/7/45	Nil	Medium	Nil	High —	48%	Low +	8/7/46	30%	Low +	Nil	Medium +	45%	Low +
err's Pink									22%	Medium —	Nil	High	60%	Low
ajestic ..	13/8/45	100%	Nil	Nil	Medium +	100%	Nil	22/8/46	100%	Nil	Nil	Medium +	100%	Low —
err's Pink									100%	Nil	Nil	High —	100%	Nil

In cauliflower, values fell to low levels in the NK (Omit P) treatment during the 1945 season but no symptoms were noticed. During the second season, however, similar values were associated with trace P deficiency. It is probable that the P level is near the threshold of deficiency.

Nitrogen. The nitrate levels, irrespective of treatment, fall as the season progresses in all crop plants studied and at the late stage of growth there is little correlation between nitrate level and visual signs of N deficiency. After certain points in the growth cycle the nitrate test cannot be used as an indicator of the nitrogen status of plants. Thus in Figs. 6-10 the hatched bands are confined to the early season when differences between treated and non-treated N plants are usually significant and values agree with the effects of N deficiency.

Visual symptoms were noted earlier in the 1946 season in the PK treatment, probably due to the wet, cold periods during the season with consequent inhibition of nitrifying bacteria and leaching out of nitrate from the soil. The lower nitrate status of the soil was confirmed by analysis and also by the observation that N deficiency was particularly severe in wet areas.

Manganese. No symptoms were observed in potato and cauliflower and tissue test values made during the 1946 season appear to be adequate. In potato and cauliflower the F.Y.M. and NP plants usually have the lowest and highest values respectively, and those receiving NPK are intermediate. There is an initial decline followed by an increase later in the season and in cauliflower this is followed by a further decrease in level.

Interrelationships of extractable nutrients.

Attention has been drawn to the critical values which can be related to the onset of visual symptoms of a mineral deficiency. Tissue test data also show that there is a close correlation between certain extractable nutrients in both potato and cauliflower, e.g. low N level with low Mg and low K levels with higher levels of Mg, Ca, PO_4, nitrate N, and Mn. These results are plotted graphically in Fig. 11. The means of the six seasonal results for K, Mg, Ca, PO_4 and NO_3 for Blocks I and III are plotted along 5 equidistant radii of a circle for the NPK, PK and NP treated potato and cauliflower. The cross hatched area in each diagram represents the threshold of deficiency as determined by visual diagnosis at centre A; the stippled portion represents the threshold as determined by data from other centres.

Potato (Majestic) 1945. The relation between the soluble nutrients K, Mg, Ca, PO_4 and NO_3 in the NPK plants is represented by a symmetrical diagram placed well outside the band showing threshold of deficiency. In the PK (Omit N) plants, however, the mean NO_3 and Mg results are within the threshold, showing the association between low N status and low Mg level. In the NP (Omit K) plants the mean K level is within the deficiency range but Ca, PO_4, and NO_3 are higher than in NPK plants.

Cauliflower. The cauliflower diagram is comparable with that of potato, but it shows that the K values are higher and N values lower than in potato. The Mg value is also lower in the cauliflower.

Tissue tests in relation to yield data.

Yield data were obtained for seasons 1945 and 1946 for both potato varieties, and for the cauliflower, in all treatments, and these were statistically treated. The

results are given in Tables VII and VIII. The symbols for the manurial treatments are as follows:

A=Nil. B=F.Y.M. C=NPK. D=PK (Omit N).
E=NK (Omit P). F=NP (Omit K).

Potato. Yields for total crop are given in Table VII. The results show that the omission of potash is the main factor depressing yields. This is in accord with the visual symptoms and the low tissue values for plants not receiving potash (Figs. 6, 7 and 8). Visual symptoms of magnesium deficiency were noted in the PK (Omit N) plots and confirmed by tissue test and ash analysis but the soil application of $MgSO_4$ to Block II, although correcting the symptoms and increasing Mg content of the foliage, did not affect the yield. The importance of N in the yield

TABLE VII.

Total yields of potato (Majestic and Kerr's Pink) for seasons 1945 and 1946. Showing treatment, total and mean yields respectively (tons per acre) and order of yields.

(*a*) *Majestic 1945.*

Manurial treatment.	A.	B.	C.	D.	E.	F.	Total.	MEAN.
Block I	3·18	11·25	12·19	8·43	7·88	3·0	45·93	7·66
Block II	6·0	9·20	11·06	9·0	10·31	5·43	51·0	8·5
Block III	5·62	9·38	10·13	10·13	7·68	5·06	48·0	8·0
Total in tons ..	14·8	29·83	33·38	27·56	25·87	13·49	144·93	
Mean in tons ..	4·93	9·94	11·13	9·18	8·63	4·5	48·31	
Order of yields ..	5	2	1	3	4	6		

Yields S.E. = 1·25. S.D. 5% = 3·92. S.D. 1% = 5·58.
At 5% point: B.C.D.E. > F. B.C.D. > A.
At 1% point: C. > AF.
Differences between block yields were not significant.

(*b*) *Majestic 1946.*

Manurial treatment.	A.	B.	C.	D.	E.	F.	Total.	MEAN.
Block I	1·69	12·19	9·0	7·31	6·75	3·0	39·94	6·66
Block II	3·94	7·31	9·56	5·25	5·44	6·56	38·06	6·34
Block III	4·88	6·94	10·69	6·38	4·69	4·68	38·26	6·38
Total in tons ..	10·51	26·44	29·25	18·94	16·88	14·24	116·26	
Mean in tons ..	3·50	8·81	9·75	6·31	5·63	4·75	38·75	
Order of yields ..	6	2	1	3	4	5		

Yields S.E. = 1·06 S.D. 5% = 3·37. S.D. 1% = 4·79.
At 5% point: C. > A.D.E.F. B. > A.F.
At 1% point: C. > A.F. B. > A.
Differences between block yields were not significant.

(c) Kerr's Pink 1946.

Manurial treatment.	A.	B.	C.	D.	E.	F.	Total.	MEAN.
Block I	5·44	14·25	10·5	10·13	12·0	3·56	55·88	9·31
Block II	6·75	12·56	14·63	10·31	13·88	5·63	63·76	10·63
Block III	6·56	13·69	13·5	11·63	11·25	4·31	60·94	10·16
Total in tons ..	18·75	40·5	38·63	32·07	37·13	13·5	180·58	
Mean in tons ..	6·25	13·5	12·88	10·68	12·38	4·5	60·19	
Order of yields ..	5	1	2	4	3	6		

Yields S.E. = 0·56. S.D. 5% = 1·78. S.D. 1% = 2·53.
At 5% point: B.C.>A.D.F. E.>A.F. D.>A.F.
At 1% point: B.>D.A.F. C.D.E.>A.F.
Differences between block yields were not significant.

of potato is shown in season 1946 in both Majestic and Kerr's Pink, the yields for the NPK treatments being significantly greater than those of PK (Omit N) treatments.

Cauliflower. The total weights of heads cut for each plot were recorded. The statistical analysis is given in Table VIII.

The results emphasize that N deficiency is the main factor in depressing head formation. Tissue test results and visual symptoms both confirm that N deficiency was severe after the first sampling dates of the two seasons, viz. July 11th, 1945, and July 15th, 1946 (Figs. 9 and 10), when nitrate N in PK (Omit N) plants was within the deficiency limit. The addition of $MgSO_4$ to Block II, although alleviating Mg deficiency, as shown by visual and chemical data, had no significant effect on the yields. Potash deficiency had no depressing effect on head formation which is in accord with the milder visual effects and higher tissue test levels recorded during the season in cauliflower as compared with potato under Omit K treatments.

TISSUE TESTS IN RELATION TO ASH ANALYSIS OF THE FOLIAGE.

The correlations between tissue test and ash analysis data for K, Mg, Ca, P and Mn are given for potato (Majestic) and cauliflower for seasons 1945 and 1946 in Figs. 12-15. The tissue test data on the ordinates in parts per million are plotted, irrespective of treatment, against ash analysis on the abscissae as percentage in dry matter. A total of 54 comparisons is possible for the three seasonal sampling times. The figures above the points indicate the number of times the chemical values coincide. The main points are as follows:

Potassium. The two methods are in agreement over the range studied. The data suggest that 1 to 2·5 per cent. K_2O in dry matter approximates to the tissue test threshold of deficiency range, viz. 25 to 50 p.p.m. K. These values are associated with the development of visual signs of the deficiency in potato and cauliflower.

Magnesium. The series of points fall on a line indicating a close correlation. In potato the critical L+ and M— tissue test range corresponds with 0·25 to 0·5 per cent. MgO, and at these levels visual signs of Mg deficiency appear. The fact that the high tissue test value (11 p.p.m.) is applicable to 1 to 2 per cent. MgO shows that the tissue test method is not sensitive at high levels.

TABLE VIII.

Total yields of cauliflower for the seasons 1945 and 1946. Showing treatment, total and mean yields respectively (tons per acre) and order of yields.

(*a*) *1945 Season.*

Manurial treatment.	A.	B.	C.	D.	E.	F.	Total.	MEAN.
Block I ..	2·62	3·55	4·75	2·16	3·80	3·95	20·83	3·47
Block II ..	4·17	4·07	4·23	2·44	3·49	3·91	22·31	3·72
Block III ..	2·93	4·38	4·04	2·81	3·36	4·41	21·93	3·66
Total in tons ..	9·72	12·00	13·02	7·41	10·65	12·27	65·07	
Mean in tons ..	3·24	4·0	4·34	2·47	3·55	4·09	21·69	
Order of yields ..	5	3	1	6	4	2		

Yields S.E. = 0·36. S.D. 5% = 0·8. S.D. 1% = 1·14.
At 5% point: B.C.E.F. > D.
At 1% point: B.C.F. > D.
Differences between block yields were not significant.

(*b*) *1946 Season.*

Manurial treatment.	A.	B.	C.	D.	E.	F.	Total.	MEAN.
Block I ..	3·52	7·09	5·63	2·11	3·97	5·60	27·92	4·65
Block II ..	3·20	5·42	4·94	3·44	5·59	7·41	30·00	5·00
Block III ..	2·19	6·0	6·04	3·53	6·20	8·06	32·02	5·34
Total in tons ..	8·91	18·51	16·61	9·08	15·76	21·07	89·94	
Mean in tons ..	2·97	6·17	5·54	3·03	5·25	7·02	29·98	
Order of yields ..	6	2	3	5	4	1		

Yields: S.E. = 0·52. S.D. 5% = 1·36. S.D. 1% = 1·93.
At 5% point: F.B.C.E. > AD. F. > C.E. At 1% point: F.B.C.E. > A.D.
Differences between block yields were not significant.

In cauliflower the tissue test deficiency threshold L+ to M− (2 to 4 p.p.m.) may be referred to approximately 0·25 per cent. MgO in dry matter.

Calcium. The two methods agree in showing that the Ca status is adequate throughout and that the M+ to H+ (130 to 200 p.p.m.) range corresponds to 4 to 8 per cent. CaO in potato whereas cauliflower has higher tissue test values for a similar total range.

Phosphorus. Tissue test data for PO_4 in parts per million, as ordinates, are referred to total values expressed as percentage P_2O_5, as abscissae.

The results for potato suggest that the two methods are related over the whole range and that 0·25 per cent. P_2O_5 corresponds to the L+ to M− (10 to 15 p.p.m. PO_4) which may be characterized by the onset of symptoms. In cauliflower

approximately 0·5 per cent. P_2O_5 corresponds to the L+ to M− tissue test range but no definite visual signs were noted.

Manganese. The soluble Mn levels in parts per thousand million (p.p.m. $\times 10^{-3}$) on the ordinate are referred to the total Mn content in the ash in parts per million dry matter on the abscissae. The quantitative data are more exact than for major elements and thus a greater scatter of points is permissible for each category.

In potato and cauliflower the results of the two methods conform to a line. A given value for soluble Mn in p.p.m. $\times 10^{-3}$ may within experimental error be referred to a similar total value in parts per million. The range covered is from approximately 20 to 70 $\times 10^{-3}$ p.p.m., soluble, and 20 to 70 p.p.m., total. Data from other centres suggest that 10 to 20 $\times 10^{-3}$ p.p.m., soluble, and 10 to 20 p.p.m., total, approximate to the deficiency level characterized by development of visual symptoms.

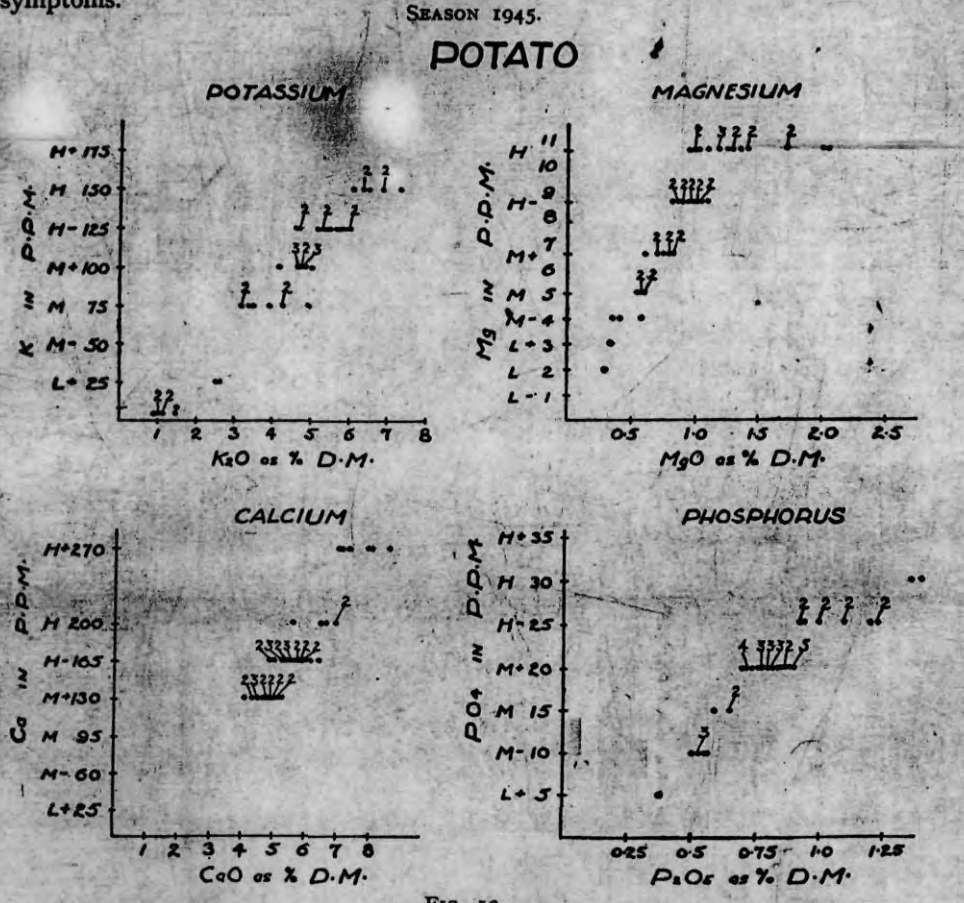

FIG. 12.

The Relation between Tissue Tests (ordinates) and Ash Analyses (abscissae) for K, Mg, Ca and P in POTATO (Majestic). Tissue test and ash analysis data given in parts per million (p.p.m.) in the extract and as percentage dry matter (D.M.) respectively. Data for the three seasonal sampling times are plotted irrespective of treatment. Season 1945. Figures above points indicate the number of times values coincide.

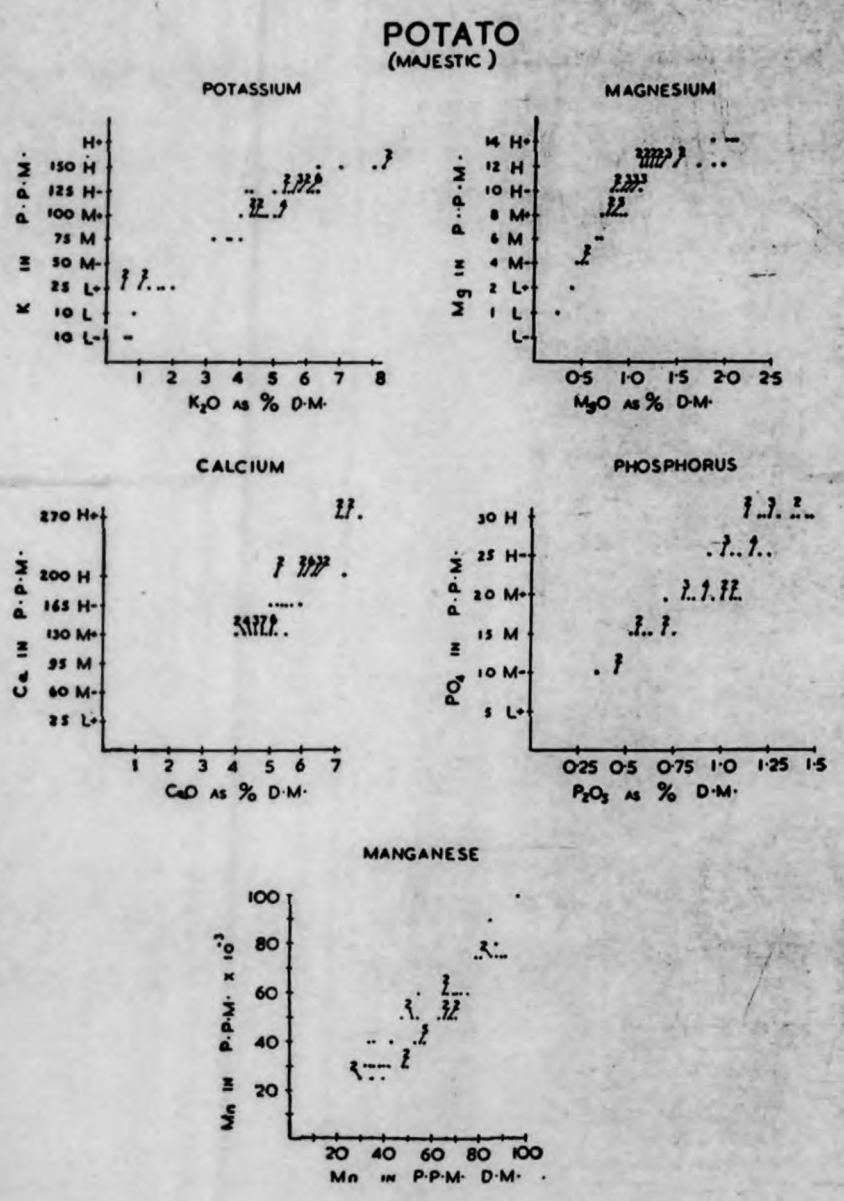

FIG. 13.

The Relation between Tissue Tests (ordinates) and Ash Analyses (abscissae) for K, Mg, Ca, P and Mn in POTATO (Majestic). Tissue test and ash analysis data given in parts per million (p.p.m.) in the extract and as percentage dry matter (D.M.) respectively. Mn in p.p.m. in the extract and dry matter. Data for the three seasonal sampling times are plotted irrespective of treatment. Season 1946. Figures above points indicate the number of times values coincide.

SEASON 1945.

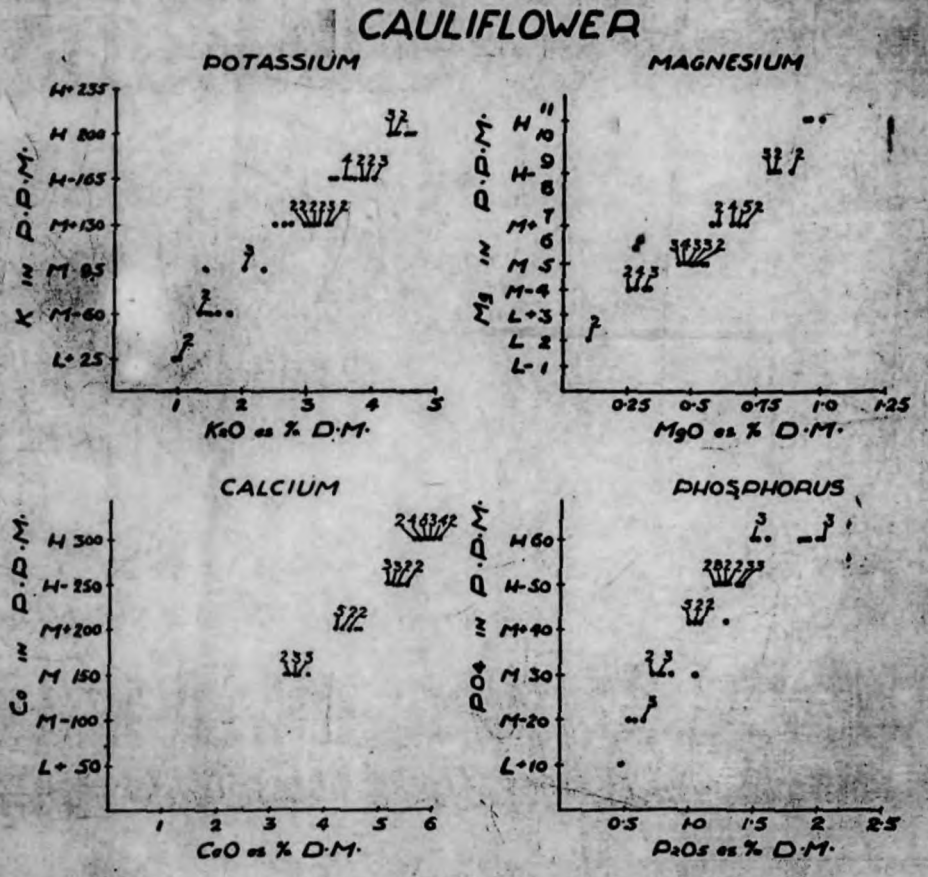

FIG. 14.

The Relation between Tissue Tests (ordinates) and Ash Analyses (abscissae) for K, Mg, Ca, and P in CAULIFLOWER. Tissue test and ash analysis data given in parts per million (p.p.m.) in the extract and as percentage dry matter (D.M.) respectively. Data for the three seasonal sampling times are plotted irrespective of treatment. Season 1945. Figures above points indicate the number of times values coincide.

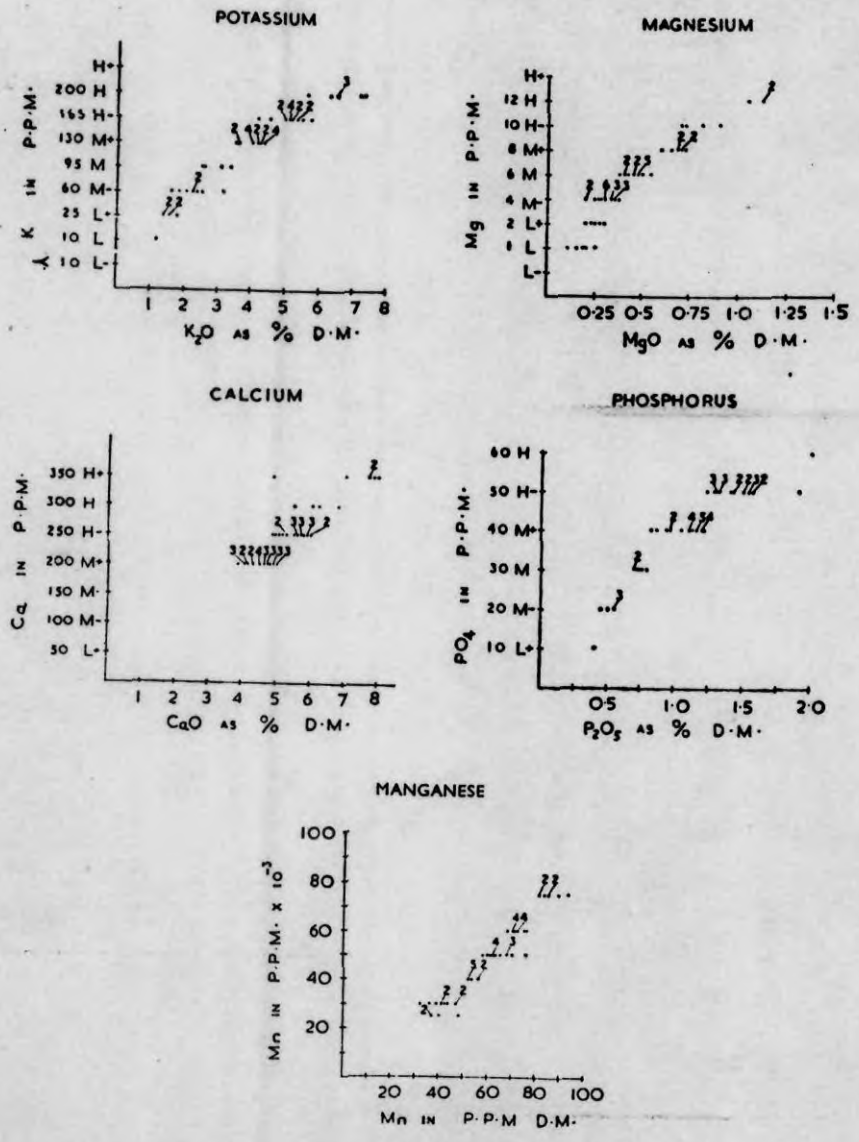

FIG. 15.

The Relation between Tissue Tests (ordinates) and Ash Analyses (abscissae) for K, Mg, Ca, P and Mn in CAULIFLOWER. Tissue test and ash analysis data given in parts per million (p.p.m.) in the extract and as percentage dry matter (D.M.) respectively. Mn in p.p.m. in the extract and dry matter. Data for the three seasonal sampling times are plotted irrespective of treatment. Season 1946. Figures above points indicate the number of times values coincide.

DISCUSSION.

A rapid tissue test technique has been developed and used in the quantitative study of the mineral status of K, Mg, Ca, Mn, PO_4 and nitrate N in potato (Majestic and Kerr's Pink) and cauliflower grown on a long term manurial trial.

In using the method a number of precautions must be taken. It is essential to standardize the field sampling procedure and to use special parts of plants that are morphologically similar. Standards for the soluble nutrients at High, Medium, and Low levels were fixed for the plants examined and the quantitative values for each level were found to vary from one crop plant to the other. It was necessary to carry out a number of preliminary determinations on healthy plants and on others showing visual signs of a mineral deficiency or toxicity to fix the quantitative values for each category. Tissue test values must be referred to the seasonal cycles for the nutrients as is shown in the data for samples taken at fortnightly intervals during the growing season. By the use of such data a deficiency or a toxicity effect can be distinguished from a seasonal trend characteristic of the crop plant and nutrient. Thus nitrate values fall to lower levels with the progress of the season in most crop plants, irrespective of treatment, and tissue test values cannot be used after certain points in the growth cycle for the diagnosis of N deficiency. The decline of nitrate N with season has also been reported by Chibnall (5), Vaidya (48) and Nightingale (36, 37) working with grass, apple and pineapple respectively.

A study of the seasonal cycles of the soluble nutrients is important for diagnostic work, e.g. medium values for certain mineral elements such as K and Mg, during early season, may suggest an impending deficiency in a susceptible crop, viz. potato and cauliflower, whereas similar results late in the season may be normal values for healthy crops, resulting from the natural downward seasonal trends characteristic of crop and nutrient. Tissue test data may be used to anticipate mineral deficiencies or toxicities prior to the development of visual symptoms.

Tissue test data show that on experimental plots the effects of the different fertilizer treatments may be recognized in the crops by this method and also that different reactions of crops growing on the same plot may be demonstrated, e.g. the K and Mg levels in potato were usually lower and higher respectively than for cauliflower grown under the same manurial treatments.

Correlation studies between tissue test results and those of the visual diagnosis method (49) were made and in these it was found possible to relate tissue test values for K, Mg, P, nitrate N and Mn, to the onset of visual symptoms. These values are regarded as threshold values for the particular deficiencies or toxicities.

Yield data for 1945 and 1946 show that K and N deficiencies are mainly responsible for the depression in yields of potato and cauliflower respectively. These results agreed with the low tissue values for K and nitrate N obtained during the growing period.

Although Mg deficiency was indicated in the PK (Omit N) treatments by visual symptoms, tissue tests and ash analysis, there were no significant differences in yields as compared with those receiving Mg, where symptoms were absent, and chemical values for Mg were higher. This demonstrates that yields do not always reflect a mineral deficiency effect.

The great value of tissue tests would be to replace with quick chemical methods the tedious and time-consuming procedures used in "total" analysis and thus several comparisons of the two procedures have been made. At Long Ashton, tissue

test data for potato and cauliflower were related to total analysis at three points in the 1945 and 1946 seasons respectively. The correlation diagrams show that there is a linear relation between the results of the two methods over the critical diagnostic range for deficiencies. At high levels found in healthy plants, however, the tissue test method does not usually distinguish different levels, due either to the fact that the 15 minutes period for the extraction of soluble nutrients is not long enough to distinguish between these high values or that over a certain total content, the proportion of less easily soluble material increases rapidly, or that at such high levels it is more difficult to differentiate colour and turbidity differences than at a lower range. For diagnostic work to determine the causes of unsatisfactory crops the point is not important and for the ranges used in both mineral deficiency and toxicity problems agreement between the two methods is close. The quick tissue tests may therefore replace the longer " total " determinations for the purpose discussed in this paper. Finally it may be stressed that the tissue test method is not only rapid and simple in operation but can be used in the field and hence is a valuable adjunct to visual and other field methods for advisory and survey work.

SUMMARY.

1. A rapid chemical tissue test method is described which has been used for the diagnosis of K, Mg, P, N and Mn deficiencies in potato (two varieties) and cauliflower, grown on a long term manurial trial.

2. The tissue test data reflected the effects of manurial treatment and also showed differences in the nutrient status of different crops grown on the same plot, e.g. potato showed a lower K content than cauliflower on plots receiving NP (Omit K) manuring.

3. The results obtained by the method showed good agreement with visual diagnosis and it was possible to fix threshold values at which deficiencies of K, Mg, P and N produced visual symptoms in a number of crops.

4. To use tissue test values for diagnostic purposes it is necessary to relate them to the seasonal cycles of the various nutrients for particular crops. Thus nitrate N was found to fall continuously as the season progressed and the nitrate test could not be used to determine N status in potatoes and cauliflower late in the season. On the other hand, with a knowledge of these cycles, the method could be used to anticipate the development of deficiencies and toxicities which only became visible at a later stage of growth.

6. Field data for potato obtained at one centre showed that K and N deficiencies caused significant reduction in crop weight. In cauliflower, N deficiency was mainly responsible for low yields. These deficiencies were indicated early in the season by chemical tissue tests.

7. A close correlation was found between the data of tissue tests and those of full chemical analysis over the range of values useful for the diagnosis of deficiencies. At the higher nutrient levels found in healthy plants the tissue test method as used did not always distinguish the differences shown by full analysis. The comparisons suggest that the tissue test method may be used to replace the longer procedure for rapid diagnostic purposes.

8. The tissue test method, because of its speed and ease of application and its suitability for use in the field, is particularly valuable for advisory and survey work.

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