CCXLVI. THE STUDY OF VARIETY AND VIRUS DISEASE INFECTION IN TUBERS OF *SOLANUM TUBEROSUM* BY THE ASCORBIC ACID TEST

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THE determination of ascorbic acid in plant material has received considerable attention, especially since modifications of Tillmans's method by various investigators has made the reduction of 2:6-dichlorophenolindophenol specific for the vitamin. Apart from detailed work on the best technique to employ for the determination, investigators have been largely concerned with comparisons of the vitamin C contents of different fruits and vegetables, the samples presumably being taken as representative of the plant under consideration, and less attention has been given so far to the variations encountered among varieties or within any one variety at different stages of growth. The present investigation followed upon an attempt to differentiate healthy and diseased tubers of varieties of *Solanum tuberosum* by a study of the oxidation-reduction potentials of the tissue and ultimately led to the use of the reagent now commonly employed in the determination of ascorbic acid. The results obtained show that it is possible not only to separate healthy tubers of a particular variety from tubers infected with virus diseases or blight, but to make a partial separation of healthy tubers of different varieties. The value of the latter observation depends, of course, upon the presumption that the tubers are free from disease, which is not valid, as far as virus is concerned, without reliable information on the source of the crop.

PRELIMINARY EXPERIMENTS

The first experiments, made with thin slices of tuber, showed that the reducing power of the tissue, measured by the time taken to decolorize definite amounts of a dilute aqueous solution of the indicator (2:6-dichlorophenolindophenol), varied considerably from point to point of the tuber. It was necessary, therefore, to adopt some method of obtaining an average value for a tuber and, since pulping is liable to hasten the oxidation of the vitamin, cores of constant diameter 11 mm. and length 15 mm. were taken by means of a cork borer and fixed parallel knives, and were found to give more uniform results. Several cores from different parts of the same tuber were used in each test and it was at once apparent that the rate of decoloration of the indicator depended upon variety and upon whether the tuber was from healthy or diseased stock.

As a means of obtaining some preliminary figures, cores from the heel, centre and rose end of each tuber were heated with 20 ml. water at 100° and the filtrate titrated with 0.0005 *M* indicator, standardized with iodine and ascorbic acid [Harris & Ray, 1933], the end-point being taken when a faint blue colour persisted for 2 min. Table I shows that the reducing power is fairly constant for

Table I. *Amount of indicator reduced after different periods of heating*

Time of heating min.	ml. indicator reduced		
	1	2	3
5	0.45	0.46	0.45
15	0.56	0.58	0.55
30	0.77	0.75	0.78

different tubers of the same variety and increases with the time of heating, whilst Table II, containing a selection of average results for tubers from different localities, indicates that the method offered considerable promise as a means of identifying variety in healthy tubers and the presence of disease in any one variety. The varieties of each pair 1 and 2, 3 and 4, 5 and 6, for example, possess tuber characteristics which make them extremely difficult to separate during the dormant season. Earlier work on this question [Smith & Robertson, 1933] had shown that the *p*-cresol-tyrosinase reaction proved to be a valuable means of identification, but there has been some change in the popularity of the varieties grown in recent years and the indophenol test seemed to offer a useful alternative or supplement.

Table II. *Amount of indicator reduced by tubers of different varieties*

Locality	...	Healthy		Virus disease
		1	2	3
		ml. indicator reduced		
Variety				
1. Eclipse		0.72	0.78	1.18
2. Majestic		0.58	0.51	0.84
3. Arran Pilot		0.39	0.72	1.08
4. Sharpe's Express		1.62	1.38	2.90
5. Kerr's Pink		1.59	1.38	2.05
6. Redskin		0.84	—	—

Although there is some evidence to show that the amount of ascorbic acid extracted from potato tubers is increased on boiling [McHenry & Graham, 1935] there are also observations to the contrary [Richardson *et al.* 1929] and it was felt desirable to reduce possible complications by using a solvent other than water and in the cold. Amongst the extracting agents tried were methyl and ethyl alcohols, acetone, and dilute hydrochloric, glacial acetic and 2% trichloroacetic acids. The alcohols were relatively poor solvents but acetone proved to be excellent for qualitative purposes, the extracts giving well-defined differences with the indicator. The end-point in titration was too indefinite, however, for quantitative purposes and attention was directed to the best method of acid extraction. The three acids mentioned above gave essentially the same results but, in view of the widespread use of trichloroacetic acid in the extraction of reducing substances from both plant and animal tissues and the essential specificity of the reaction with indophenol for ascorbic acid at pH 2-3, it was decided to carry out further work with this acid. On titrating an extract of potato tuber with the indicator, the colour is at first discharged but a faint pink colour persists at the end-point; the decolorizing effect of the trichloroacetic acid is negligible if the titration is carried out expeditiously.

Extraction with trichloroacetic acid

Three cores, taken from each tuber as previously described, were weighed and ground up with sand and 20 ml. of the acid, and the filtrate was made up to a definite volume. The residue was extracted a second and third time and the

results in Table III show that at least two extractions were necessary to obtain a maximum figure for "ascorbic acid". This procedure, applied to three tubers of each of twelve varieties from two sources, gave the average results contained in Table IV, from which it is obvious that, taking differences greater than three times the standard error as significant, many important separations can be effected. For example, there is no difficulty in separating Redskin from Kerr's Pink or Majestic from Eclipse or Arran Pilot from Sharpe's Express to mention only a few cases in which varieties are liable to be confused during the dormant season.

Table III. *Extraction of "ascorbic acid" from Arran Banner (3-4 months after harvest)*

No. of extractions	mg. "ascorbic acid"/ 100 g. tuber
1	6.42 $\pm$ 0.18
2	8.39 $\pm$ 0.16
3	8.38 $\pm$ 0.16

Table IV. *"Ascorbic acid" content of different varieties (after 3-4 months' storage)*

Variety	"Ascorbic acid" mg./100 g.	Variety	"Ascorbic acid" mg./100 g.
1. Redskin	5.91 $\pm$ 0.09	7. Eclipse	7.67 $\pm$ 0.23
2. Doon Star	6.09 $\pm$ 0.13	8. Great Scot	7.71 $\pm$ 0.16
3. Arran Pilot	6.59 $\pm$ 0.11	9. Arran Banner	7.86 $\pm$ 0.17
4. Majestic	6.85 $\pm$ 0.12	10. Sharpe's Express	8.15 $\pm$ 0.19
5. Dunbar Cavalier	7.21 $\pm$ 0.20	11. Kerr's Pink	8.21 $\pm$ 0.18
6. Gladstone	7.58 $\pm$ 0.12	12. King Edward	8.44 $\pm$ 0.28

Dehydroascorbic acid. There is evidence that in many plants a considerable proportion of the ascorbic acid may be in a reversibly oxidized state and that a reliable estimate of its amount can be obtained only after a suitable reduction of the tissue extract. To effect this, the extract is treated for 15 min. with hydrogen sulphide and allowed to stand overnight and the excess sulphide driven off by passing nitrogen through the solution before titration. A further precaution, commonly adopted to get rid of interfering reducing substances, is to treat the extract with a 20 % solution of mercuric acetate and filter, before the subsequent sulphide treatment [Van Eekelen & Emmerie, 1936]. These steps were carried out with two tubers of each of three varieties, soon after the plants had reached maturity, and the results are given in Table V. There is a tendency for the double extraction alone to give the higher results but it is doubtful if the differences between the figures for each variety can be regarded as significant. It would appear, therefore, that, when the tuber is examined immediately after harvest, the result obtained by the indophenol titration of the trichloroacetic acid extract is a measure of the ascorbic acid in the tuber. That this may not be the case after the tubers have been stored for some time is suggested by a comparison of the figures in Table V with those in Table IV obtained after 3-4 months' storage. But the principal object of this investigation was to find a suitable rapid

Table V. *Ascorbic acid (mg./100 g. tuber) obtained by different treatments*

Variety	...	Eclipse	Arran Pilot	Sharpe's Express
Double extraction		22.0	16.7	24.6
H ₂ S treatment		19.8	15.8	22.8
Mercuric acetate		19.4	16.3	23.2

means of identifying variety or separating healthy from diseased tubers, for which purpose even a simple double extraction was too laborious.

Modified method for routine purposes. In order to reduce the time required for a comprehensive examination of various samples, the cores were simply shaken for 1 min. with 2 ml. of 2% trichloroacetic acid and the mixture titrated with indicator. The results obtained by this method were much smaller but the differences between varieties were still quite significant (see Table VII) and important differences were always obtained between healthy and diseased stock.

Factors concerned in concentration of "ascorbic acid"

It was possible that several factors might affect the results obtained as above and the following points were investigated:

(1) *Tuber weight.* Twelve tubers of each of four varieties, varying in weight from 40 to 400 g., were examined, but the variation within any variety was no greater than that usually found between individual tubers of approximately the same size.

The standard error of the means actually lay between 3 and 7% so that the results were obviously not affected by tuber weight.

(2) *Environment.* Under this head may be considered climate and soil conditions. The former may be regarded as constant for the centres in which the crops were grown; the latter may be regarded as determined by the physical and chemical properties of the soils in question and the management. In the maturity investigation, described below, the soils at the four centres varied considerably and might be described as a sandy loam, a medium loam, a heavy loam and a garden soil, and the average pH values varied from about 5.0 to 7.0. In spite of these differences, the indophenol values did not differ by more than experimental error. Furthermore, the results for healthy tubers given in Table VII are the average figures for two tubers from each of five sources and the extent of the variations would appear to rule out environment as a factor of any importance.

The question of manuring was also examined. In 1936, plots of variety Golden Wonder were manured at rates varying from half to four times the normal, but the tubers from the different plots all gave results which did not differ from these for the unmanured crop by more than experimental error. Tubers of variety Great Scot were also grown in pots, the pH of the soil being adjusted to the levels 5.4, 5.9, 6.4, with and without addition of a normal dressing of fertilizers, but the results showed no significant differences. Similarly in 1937, plots of the variety Eclipse were given a basal dressing of nitrogen and phosphorus and increasing amounts of potassium up to four times the normal, but again the results for the tubers from the different plots did not differ beyond experimental error. The same experiment carried out in pots yielded almost identical figures. It appears, therefore, that the amount of reducing material in the tuber is unaffected by soil type or ordinary manurial treatment. Ott [1937] found small differences as a result of various manurial treatments.

(3) *Maturity and storage.* This factor, which would be of considerable importance in the use of the test for deciding variety or differentiating healthy and diseased stock, was studied by marking two plants of each of seventeen varieties of healthy stock in each of four centres in the neighbourhood of Edinburgh at the early stages of growth, and taking two tubers from each plant for examination at intervals of about a month before normal harvesting. Further random samples were taken at the time of harvest and stored for examination after 1 month. Four varieties were further examined at intervals of 8 weeks after

storage (*a*) in a store at 3–7° and (*b*) in the laboratory at 12.5–18.5°. Samples of these varieties were also stored exposed to daylight in a manner commonly adopted on the farm to induce greening for seed purposes and examined after about 4 months when fully “greened”.

It was not possible to examine tubers of size less than about 1½ in. long, in other words, before the beginning of August, and the results showed that usually a maximum value was then obtained. At the beginning of September, and again a month later, the value was considerably smaller. It was also noticeable that the variation within variety decreased considerably as maturity was reached. During cold storage, the value remained fairly constant until December and then decreased, sharply at first and then more gradually. Under the conditions of laboratory storage, the rapid decrease in the value occurred earlier. A selection of these results for four varieties is given in Fig. 1 and illustrates the above observations; the four points marked “G” indicate the average results obtained with tubers which had been allowed to become green by exposure to light during storage and show that the values are not significantly affected by this treatment. The above results, which confirm the observations of Pett [1936] and Olliver [1936], reveal the importance of time and temperature of storage in the comparison of tubers by this modified procedure of estimating ascorbic acid.

Fig. 1. Date and time of storage in months. — Cold store. — Laboratory.
× Eclipse. • Majestic. ○ King Edward. □ Dunbar Cavalier.

(4) *Sprouting*. The decrease in the results during storage may be due either to the oxidation of the ascorbic acid or to its movement to the sprout from the parent tuber, and the distribution of reducing substance between tuber and sprout was investigated. For this purpose, a large number of tubers of three varieties was placed in shallow trays at the beginning of April and covered with sand, which was kept moist. Samples of four tubers of each variety were examined at weekly intervals, and, when they were large enough, the sprouts were cut into small pieces and composite samples taken for extraction.

The average results, plotted in Fig. 2, show that there is a slight increase in the concentration of ascorbic acid in the tuber at time of sprouting and then a decrease, and that the sprouts are at first relatively rich in ascorbic acid. At the 7th and 8th times of sampling, the tubers and sprouts were weighed before examination so that the total quantities of ascorbic acid present might be estimated. By calculating the results to a basis of 100 g. tuber (Table VI) it may be seen that there is actually a net loss of ascorbic acid from tuber and sprout

amounting to about 0.2 mg. between the 7th and 8th weeks. (The figures for Arran Banner may be abnormal in that the relative weight of sprouts was smaller in the 8th than in the 7th week of sampling.) It would seem, therefore, that the increase in ascorbic acid in the sprouts is readily accounted for by the decrease in

Fig. 2. — — — Sprouts. — — — Tubers.
× Eclipse. ○ Arran Banner. • Majestic.

the parent tuber at an advanced stage in sprouting. The observations are inadequate, however, to account for the apparent increase in the amount of ascorbic acid in tuber and sprout at the commencement of sprouting. Pett [1936] found a very large increase in the concentration of vitamin in the tuber when sprouting started, followed by a rapid decrease, all in the space of about a fortnight, but the period of storage before experiment was probably much shorter than was the case here.

Table VI. *Ascorbic acid in tuber and sprout*

Variety	...	Eclipse		Arran Banner		Majestic	
		Tubers	Sprouts	Tubers	Sprouts	Tubers	Sprouts
7th week (wt. in g.)		326	70	376	52	330	67
mg. ascorbic acid		17.8	2.6	14.2	2.9	11.0	2.4
mg. ascorbic acid/100 g. tuber		5.46	0.80	3.78	0.77	3.33	0.73
8th week (wt. in g.)		396	95	347	36	378	95
mg. ascorbic acid		20.6	4.4	12.5	2.1	10.5	3.7
mg. ascorbic acid/100 g. tuber		5.20	1.11	3.60	0.61	2.78	0.98
Gain		-0.26	+0.31	-0.18	-0.16	-0.55	+0.25
Net gain			+0.05		-0.34		-0.30

(5) *Disease*. The effect of disease on the test was studied by examining samples of six tubers certified as affected with different diseases after 1 month's storage. With only one or two exceptions, tubers infected with mild and severe mosaic gave the same results within experimental error, and a mean figure has been given for mosaic. The number of cases of leaf roll was more limited but the results do not differ much from those under mosaic. In both cases the amount of

reducing material is significantly greater (with one exception) than in the healthy tuber after the same storage period—an observation of great value as offering a simple means of testing the presence of virus disease in tubers. It is of interest, although of no apparent practical importance, to find that tubers infected with blight (*Phytophthora infestans*) contain less reducing substance than healthy tubers. The results are collected together in Table VII, those for healthy tubers being the means for ten tubers after 1 month's storage.

Table VII. *Amount of indophenol (ml.) reduced by healthy and diseased tubers*

Variety	Healthy	Mosaic	Leaf roll	Blight
Arran Pilot (1)*	1.48 ± 0.08	2.10 ± 0.05	—	1.09 ± 0.02
Majestic (2)	1.54 ± 0.06	2.37 ± 0.09	2.15 ± 0.08	1.36 ± 0.05
Doon Star	1.66 ± 0.10	—	2.15 ± 0.08	1.39 ± 0.04
Ninetyfold	1.79 ± 0.05	—	2.42	1.32
Great Scot (3)	1.88 ± 0.06	2.30 ± 0.10	2.29 ± 0.05	1.47 ± 0.04
Dunbar Cavalier (4)	1.95 ± 0.04	2.33 ± 0.11	2.24 ± 0.10	1.44 ± 0.04
Immune Ashleaf (1)	1.95 ± 0.14	2.55 ± 0.15	2.53 ± 0.10	—
Redskin (5)	1.96 ± 0.12	2.63 ± 0.14	—	1.33 ± 0.03
King George	1.99 ± 0.04	—	—	—
British Queen	2.17 ± 0.04	—	—	1.17
Arran Banner (3)	2.22 ± 0.07	3.47 ± 0.11	2.87 ± 0.05	1.66 ± 0.03
Kerr's Pink (5)	2.33 ± 0.07	—	—	1.63 ± 0.05
Gladstone (4)	2.43 ± 0.05	3.40 ± 0.14	3.06 ± 0.09	1.99 ± 0.05
Eclipse (2)	2.45 ± 0.07	3.41 ± 0.09	—	2.03 ± 0.08
Sharpe's Express (1)	2.66 ± 0.07	3.33 ± 0.10	—	1.64 ± 0.08
Duke of York	2.66 ± 0.03	2.85 ± 0.15	—	—
King Edward (4)	2.73 ± 0.08	3.20 ± 0.14	—	1.71 ± 0.06

* Varieties liable to be confused carry the same numbers.

(6) *Season.* The only rigid comparison for seasonal effect is for the variety Eclipse at time of lifting in 1936 and again in 1937. In each case the reducing power of three cores was equivalent to 3.1 ± 0.2 ml. indophenol. The variation during storage after harvest excluded comparison of the figures available for other varieties, but the relative amounts of reducing substance were essentially the same for a series of twelve varieties collected in 1935 and in 1936, so that the same varietal differences were found in each season. Varieties, whose tubers are often difficult to distinguish, are appropriately marked in Table VII and, in addition to those previously mentioned, include Great Scot and Arran Banner, Dunbar Cavalier, Gladstone and King Edward, Immune Ashleaf and Sharpe's Express.

DISCUSSION

The most significant feature of the above results is that the ascorbic acid content of potato tubers is extremely variable. The variation seems to be independent of size and weight of tuber, soil properties and possibly season, and to be due mainly to variety, health and storage conditions, particularly the duration of storage. It is possible that the arbitrary rapid routine method adopted, of extracting three cores of tuber tissue for a short period with trichloroacetic acid and titrating the mixture with the indophenol indicator, does not give a constant fraction of the total ascorbic acid present because there are differences in the texture of the tissues of different varieties. There are, unfortunately, no data available for the total ascorbic acid and the simple extract reducing value of material after the same storage periods; nor are there figures to show whether the decreases in total ascorbic acid and reducing value run parallel during storage. Although it would seem, from the results in Table V, that the ascorbic acid is

present entirely in the reduced form in mature tubers freshly harvested, it is possible that, after a period of storage (Tables III and IV) or during sprouting (Table VI), part of the ascorbic acid is in the reversibly oxidized form and is not determined by double extraction without reduction. Certain discrepancies in the order of results for different varieties (Tables IV and VII) suggest that the total and single extraction methods are not strictly comparable, but nevertheless several varietal differences are quite significant by each method, and the rapid routine method provides a most useful means of discriminating variety under similar storage conditions. Of possibly greater importance is the value of the test in separating healthy from diseased stock, again under similar storage conditions. The test would, therefore, seem to afford useful assistance to growers in the selection of stock seed for the next season's crop. It is also of interest to observe that there appears to be a general correlation between the susceptibility of a variety to virus disease and its content of ascorbic acid. In this respect, the results in Table IV are very suggestive, varieties 1-5 being susceptible and 6-12 resistant to infection. In Table VII, however, the varieties Great Scot and Duke of York are out of order. McIntosh [1935] has drawn attention to the possibility of measuring resistance to virus disease by chemical means and emphasized the great importance of such a test in controlling the disease. It is possible that an extension of the present investigation might help to achieve this purpose.

SUMMARY

A large number of healthy and diseased potato tubers from the 1935, 1936 and 1937 crops have been examined for ascorbic acid by the usual method of extraction with trichloroacetic acid and titration with 2:6-dichlorophenol-indophenol and by a modification suitable for rapid routine purposes. Both methods reveal that considerable variations exist in the reducing material present in tubers and that the important factors involved are variety, freedom of the stock from virus disease and duration of storage. The measurement in fact provides a means of identifying variety in healthy tubers and the presence of disease in any one variety. Such factors as tuber size, season and environment, including soil conditions and manuring, do not seem to influence the results.

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