and petiole, the interveinal tissue did not reach tritium equality with major veins, the stem, or the external solution. Water of the interveinal tissue maintained a stable tritium content at 60 to 70% of external concentration for long periods. The pattern of results obtained was similar in sunflower and tobacco.

Tritium content of leaf-tissue water vapor transpired by the canopy of an intact sunflower plant raised from seed in THO and treated with a stream of dry air inside a closed chamber increased over a 12-hour period. At the end of this period, interveinal tissue water and transpired vapor reached at least 95% of the external tritium concentration about the roots. It was shown that the lack of equilibrium in interveinal tissue should be attributed to exchange of tritium between the leaf and the surrounding unlabeled water vapor of the atmosphere. The results reported here contradict the hypothetical concept that a sizable fraction of tissue water is inaccessible for turnover.

Acknowledgment

The authors are grateful for the support given to a research program partly reported here by the United States Army Electronic Proving Grounds Technical Program Contract DA-36-039-SC903-34, and by Western Regional Research Project W-67.

Citric-acid Induced Loss of Rigidity in Potato Tuber Slices

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While examining the action of enzymes at pH 3.5 to 4.0 upon the breaking strength of slices of potato tubers (cf. 13) we observed that in citrate-phosphate buffer alone the slices became flaccid. This is surprising in view of the fact that such a buffer has been used commonly in studies with plant tissues and tissue preparations, usually at higher pH, however.

Citric and other Krebs-cycle acids have been found to stimulate the respiration and other metabolic activities of plant parts (8, 9, 14, 23), but in such studies few, if any, comments are made concerning the physical properties of the tissues. However, some of these acids have been reported to have deleterious effects (8, 11, 21).

In the work described below slices of potato tuber were mounted as cantilever beams following various treatments. Their deflection under their own weight was observed and interpreted using the theory for an elastic, uniformly-loaded cantilever beam (20).

Materials and Methods

All of the chemicals used were reagent grade and distilled water was further purified by passing it through a reagent-grade, mixed-bed resin column. Potato tubers were purchased at local markets and were stored in a refrigerator at about 5°C. Some hours to a day or so before being used they were placed in distilled water in the refrigerator. Tubers

Literature Cited

similar to, if not actually of, the Russet Burbank variety were used since they responded better than the few other varieties tested.

All treatments were carried out at 30° ± <0.1° unless stated otherwise. Slices were cut 1 mm thick using a hand microtome and were trimmed to a uniform 13 mm × 28 mm. A small corner portion was removed from each slice to index it so that it could be oriented repeatedly in the clamp in the same manner.

Before treatment the slices were washed for about 15 minutes in running cold tap water and were weighed individually after uniform blotting for 10 seconds with bibulous paper. After treatment each slice was blotted, weighed, and one end was fastened horizontally in a plastic clamp with spring-loaded jaws. Fresh slices assumed a horizontal position; wilted ones sagged. The downward deflection of the free end of the slice was noted. In some cases both the x and y positions of the end of the slice were noted; in earlier experiments only the y position. A stop permitted each slice to be inserted uniformly 4 mm into the jaws.

Each lot of 5 slices was placed in 40 to 50 ml of solution. The volume was always constant for any one experiment. Gentle stirring was provided by shaking.

Since we are dealing with uniformly-loaded, elastic cantilever beams the equation describing their deflection was examined for its possible applicability.

The downward deflection, y, of the free end of such a beam is related to other properties as follows (20):

\[-y = \frac{a x^4}{8 EI},\]

where q is the loading rate, x is the total projected length of the beam on the x axis, E is the modulus of elasticity and I is second moment of area (moment of inertia of cross section). In our experiments q = weight/length of slice = w/l. EI is a measure of the rigidity of the slice, and, since it is difficult to assess I for a potato slice, these 2 were not separated in treating the data. For any beam of given material and dimensions EI is usually presumed to be constant, and characteristic of the material being tested. However, in our case the interaction of the elastic cell walls with the pressure exerted by the cell contents determine its apparent magnitude. Hence, the rigidity would be expected to be influenced by the treatments which influence either the elastic properties of the wall or the hydrostatic pressure of the cell contents.

The equation for EI was difficult to use directly since the fresh, turgid, slices usually showed no deflection and in some cases even showed small upward curvatures. Under these circumstances EI assumed absurd values. Hence, the reciprocal was used:

\[-\frac{1}{EI} = \frac{8 y}{w x^4} = - MF\]  

MF is defined as the negative of the moment of flaccidity or the reciprocal of the rigidity.

**Experimental Results**

After most of the experiments had been completed the possible applicability of the cantilever beam theory was appreciated. Additional experiments were undertaken for the purpose of evaluating the application of this model to potato slices. Slices were washed with H₂O and were treated for 1 hour with 0 to 1.0 M sucrose solutions. For deflections from 0 to -1.1 cm the mean values for x (distance from jaws) for each were associated with low standard deviations. However, for deflections greater than -1.4 cm the observed values for x were somewhat less reproducible and the slices were rather wilted. The larger standard deviations for these latter slices result from 2 conditions: A) some slices in a sample lose turgor more readily than others and B) the wilted slices are somewhat less elastic than turgid ones. Hence, they tend to come to rest in various random positions.

The observed curvature of slices was compared with that predicted by the theory for a uniformly-loaded, elastic cantilever beam using a graded series of sucrose solutions. The correspondence between the 2 was good to about 0.32 M (y = -1.3 cm), but was less satisfactory in higher concentrations (y ≥ -1.7 cm). This theory was never intended to be applied to deflections as large as these. Yet it gave a reasonably good description of the observations. Uncertainties arising from variability in tissues appeared to be at least as serious as inadequacies of the mathematical model.

In early experiments only values for deflection (-y) were noted. However, it was found (fig 1) to be a reasonably safe procedure to compute MF values from the observed deflections, along with observations on weight and length. This was done for the experiments reported here, unless stated otherwise. Both x and y were observed after the value of the cantilever beam model was recognized.

Note that the flaccidity is presented in the figures as a semi-log plot of MF + 2. This was done solely to facilitate displaying data ranging from 0 to >1000. Care should be taken in interpreting this plot, particularly at low values of MF.

Responses to 0.01 M citrate-phosphate buffer are summarized in table I. A 0.1 M citrate-phosphate buffer elicited very similar responses, except for the fact that the fresh weight and length decreased from the outset (cf. tables I and II). Analysis of variance showed that for both concentrations the duration of the treatment with buffer had a statistically significant (P<0.01) effect upon the rigidity of the tissues, despite a rather large, but statistically non-significant, day to day variation. In both cases the increase in moment of flaccidity was not statistically significant for about the first 2 hours.

In all these buffers tended to increase in pH during the experiments. In some cases the solutions were replaced every hour to minimize such a change. When this was done the increase in pH was reduced to about 0.1 unit in the 0.1 M and about 0.2
Table I. Influence of 0.01 M Citrate-Phosphate Buffers upon the Flaccidity and Fresh Weight of Potato Slices

The citric acid concentration was 0.0053 M. Initial pH was 3.6 to 3.8. The data are the summary of 8 experiments.

<table>
<thead>
<tr>
<th>Time treated hours</th>
<th>Moment of flaccidity (g^{-1} \text{cm}^{-2})</th>
<th>Decrease in wt mg/slice</th>
<th>Wt decrease for each one-half hr interval mg/slice per hr</th>
<th>Decrease in length cm \times 100/slice</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.03</td>
<td>((364 \pm 7.2)^{**})</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>0.5</td>
<td>0.09</td>
<td>((-8.9 \pm 11.9)^{*})</td>
<td>-18</td>
<td>(-5.1 \pm 2.9^*)</td>
</tr>
<tr>
<td>1</td>
<td>0.19</td>
<td>((-1.8 \pm 12.3))</td>
<td>14</td>
<td>(-5.5 \pm 3.1)</td>
</tr>
<tr>
<td>1.5</td>
<td>0.50</td>
<td>((20 \pm 12.7))</td>
<td>44</td>
<td>(-5.1 \pm 3.0)</td>
</tr>
<tr>
<td>2</td>
<td>5.3</td>
<td>((59 \pm 13.3))</td>
<td>78</td>
<td>(4.2 \pm 1.5)</td>
</tr>
<tr>
<td>2.5</td>
<td>107</td>
<td>((88 \pm 12.9))</td>
<td>58</td>
<td>(12.1 \pm 3.4)</td>
</tr>
<tr>
<td>3</td>
<td>691</td>
<td>((108 \pm 11.3))</td>
<td>40</td>
<td>(20.5 \pm 2.9)</td>
</tr>
</tbody>
</table>

* Mean ± se of change in weight or length, as the case may be. The se's were calculated from the averages for each experiment. The values shown here are the means for the combined data from all experiments.

** Average initial fr wt ± se.

unit in 0.01 m buffer. Without such replacement the pH increased by as much as 0.3 unit in the more concentrated buffer and by as much as 0.4 unit in the more dilute one.

The pattern of weight loss, table I, appears different from that for increase in flaccidity, cf. figure 1. Note that the rate of weight loss reached a maximum at 2 hours, just prior to the first marked increase in MF. The loss in weight during the course of the experiment was not due merely to repeated blotting of the slices. Higher pH's in 0.1 m citrate-phosphate buffer were found to have much less effect (or a much slower effect) upon the rigidity of potato slices (see fig 2).

Since in the usual citrate-phosphate buffer the amount of citric acid added decreases with increasing pH while the amount of phosphate increases, the influence of phosphate concentration, and pH were examined independently. A series of buffers were studied in which the concentration of citric acid (plus its salts) was constantly 0.0053 m and various pH values were obtained by using appropriate volumes

Fig. 1. Moment of flaccidity \(-\frac{1}{E_1}\) and changes in fresh weight, of potato tuber slices in sucrose at various concentrations. \(X\) = both \(x\) and \(-y\) observed and used to compute MF. \(Q\) = MF values computed from \(-y\) values only (see text). Broken line = fresh weight; solid lines = MF ± se.

Fig. 2. Changes in flaccidity and fresh weight of potato slices in 0.1 m citrate-phosphate buffer at various initial pH's. \(\circ\) = pH 3.4; \(\bullet\) = pH 4.4; \(\oplus\) = pH 5.4. Broken line = fresh weight; solid lines = MF ± se.
of 0.1 m Na₂HPO₄. The total phosphate concentration was 0.0048 m, 0.0088 m and 0.0125 m at pH 3.5, 4.5 and 5.5, respectively. Another similar series was prepared in which the pH was adjusted by using NaOH. The results with the 2 series of buffers were essentially the same, both for changes in flaccidity and fresh weight, i.e. phosphate, per se, had no measurable effect upon the results. The influence of these 0.01 m buffers upon changes in weight, flaccidity and length of slices was studied in detail also at higher pH's viz., 4.4 to 4.7 and 5.5 to 5.7. The results obtained were consistently as in figure 2, except that the slices increased in weight at first, cf. table I. This increase at these higher pH's was approximately twice that at pH 3.7 at the same concentration and was followed by a loss in weight as in table I. The observed changes in MF and length were not statistically significant.

The differences in weight changes during the initial period of treatment in the 2 concentrations of buffer led to concern for the osmotic concentration of the solutions. The freezing point of the 0.1 m, pH 3.7 buffer showed it to be equivalent to 0.23 m sucrose.

Weight and MF changes elicited by 0.1 m citric acid-phosphate buffer and by solutions of sucrose are shown in table II. These data are from 2 separate experiments for which the results were very similar. In one case both deflections (-y) and x values were observed. In sucrose solutions the slices reached an equilibrium in both rigidity and fresh weight in about 1 hour. However, in the buffer the slices continued to lose weight for at least 3 hours and became flaccid. Obviously the osmotic concentration of the pH 3.7 buffers, especially the 0.01 m one, is not sufficient, per se, to explain the observed loss in rigidity.

Table III summarizes a comparison of citric and phosphoric acid buffers. During the course of the experiment the pH was adjusted at either half-hourly or hourly intervals to minimize changes. As a consequence the pH 3.5 buffers actually varied from pH 3.5 to 3.9, the pH 4.5 buffers from pH 4.5 to 5.0, and the pH 5.5 solution from pH 5.5 to 5.8. The results of 2 experiments, each with 5 slices per treatment are included. Note the marked difference in response to these 2 buffers. On the other hand 0.005 m succinic acid produced responses very similar to those obtained with citric acid (table IV). These data represent 3 separate experiments, each with 5 slices per treatment.

It was observed repeatedly that the induction of flaccidity by citrate buffer was inhibited by calcium ions. The results of 3 experiments are summarized in figure 3. In each experiment 5 slices from a single potato tuber were shaken in 25 ml of each of the solutions. Both x and y positions were observed for the free end of the slices. For each treatment 3 other slices from the same potato tuber were used for respiration measurements. Each slice was cut into 12 pieces and suspended in 2 ml of deionized H₂O in a Warburg flask. When temperature equilibrium

Table II. Comparison of the Effect of 0.1 m Citric Acid-Phosphate Buffer, pH 3.7, and Sucrose Solutions upon the Fresh Weight and Flaccidity of Potato Slices

<table>
<thead>
<tr>
<th>Time treated (hr)</th>
<th>H₂O Moment of flaccidity g⁻¹ cm⁻²</th>
<th>Buffer Moment of flaccidity g⁻¹ cm⁻²</th>
<th>Sucrose 0.2 M Moment of flaccidity g⁻¹ cm⁻²</th>
<th>H₂O Loss in weight mg/slice</th>
<th>Buffer Loss in weight mg/slice</th>
<th>Sucrose 0.2 M Loss in weight mg/slice</th>
<th>Sucrose 0.4 M Loss in weight mg/slice</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.02</td>
<td>0.01</td>
<td>-0.02</td>
<td>(350)*</td>
<td>(350)*</td>
<td>(351)*</td>
<td>(357)*</td>
</tr>
<tr>
<td>1</td>
<td>0.04</td>
<td>0.41</td>
<td>0.13</td>
<td>61</td>
<td>-18</td>
<td>45</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>0.18</td>
<td>2.1</td>
<td>0.15</td>
<td>57</td>
<td>-24</td>
<td>83</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>...</td>
<td>63</td>
<td>0.16</td>
<td>61</td>
<td>...</td>
<td>127</td>
<td>22</td>
</tr>
</tbody>
</table>

* Initial weight, mg/slice.

Table III. Comparison of the Effect of Citric Acid-NaOH Buffer with Phosphoric Acid-NaOH Buffer upon the Flaccidity and Weight of Potato Slices

<table>
<thead>
<tr>
<th>Time treated (hr)</th>
<th>Citrate buffer pH 3.5 Moment of flaccidity g⁻¹ cm⁻²</th>
<th>pH 3.5 Phosphate buffers</th>
<th>pH 4.5 Phosphate buffers</th>
<th>pH 5.6 Phosphate buffers</th>
<th>Citrate buffer pH 3.5 Moment of flaccidity g⁻¹ cm⁻²</th>
<th>pH 3.5 Phosphate buffers</th>
<th>pH 4.5 Phosphate buffers</th>
<th>pH 5.6 Phosphate buffers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.06</td>
<td>0.03</td>
<td>-0.07</td>
<td>0.03</td>
<td>(367)*</td>
<td>(354)*</td>
<td>(352)*</td>
<td>(353)*</td>
</tr>
<tr>
<td>1</td>
<td>-0.32</td>
<td>0.06</td>
<td>-0.04</td>
<td>-0.05</td>
<td>8</td>
<td>-1</td>
<td>-15</td>
<td>-13</td>
</tr>
<tr>
<td>2</td>
<td>1.9</td>
<td>0.10</td>
<td>0.08</td>
<td>-0.07</td>
<td>50</td>
<td>6</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>2.5</td>
<td>0.40</td>
<td>0.18</td>
<td>0.10</td>
<td>-0.07</td>
<td>70</td>
<td>20</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>0.65</td>
<td>0.45</td>
<td>0.13</td>
<td>-0.05</td>
<td>88</td>
<td>33</td>
<td>17</td>
<td>19</td>
</tr>
</tbody>
</table>

* Initial weight, mg/slice. Copyright © 1965 American Society of Plant Biologists. All rights reserved.
Table IV. Influence of Succinic Acid-NaOH Buffers upon the Flaccidity and Weight of Potato Slices

The succinic acid concentration was 0.005 M. x Values were estimated from observed deflections.

<table>
<thead>
<tr>
<th>Time treated hr</th>
<th>Moment of flaccidity g(^{-1}) cm(^{-2})</th>
<th>Loss in wt mg/slice</th>
<th>3.5</th>
<th>4.5</th>
<th>5.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>3.5</td>
<td>4.5</td>
<td>5.5</td>
<td>(367 ± 7.2)*</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0.05</td>
<td>0.09</td>
<td>0.04</td>
<td>-14</td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td>0.13</td>
<td>0.16</td>
<td>0.07</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0.20</td>
<td>0.15</td>
<td>0.11</td>
<td>24</td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td>0.30</td>
<td>0.25</td>
<td>0.17</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.22</td>
<td>0.41</td>
<td>0.31</td>
<td>83</td>
</tr>
<tr>
<td>2.5</td>
<td></td>
<td>0.38</td>
<td>1.6</td>
<td>0.56</td>
<td>104</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.425</td>
<td>38</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

* Initial weight, mg/slice, ± se.

Fig. 3. Moment of flaccidity and respiration of potato slices as influenced by citrate buffer in the presence of various salts. Broken lines = oxygen uptake; solid lines = MP + 2. ○ = H\(_2\)O; △ = 0.005 M citrate buffer, pH 3.5; X = buffer + 0.005 M CaCl\(_2\); □ = buffer + 0.005 M MgCl\(_2\).

had been reached, 0.5 ml of a solution as used for flaccidity measurements, except 10 times as concentrated, was tipped into the main compartment of the flask. This produced treatments as for the flaccidity measurements, except that the solutions were twice as concentrated, but only one-half the volume of solution was used for each slice. Hence, the buffering capacity and pH were comparable in the 2 series of measurements. Shortly after tipping in the buffer, or buffer + salt solution, the flask was closed and oxygen uptake was observed for 2 hours.

The pH increased in all cases during the treatments. In the citrate buffer alone the increase was about 0.6 pH unit; in buffer plus CaCl\(_2\) about 0.4 pH unit; and in buffer plus MgCl\(_2\) no change was observed in 1 case and in the other cases an increase of about 0.6 pH unit was obtained.

The similarity in responses in H\(_2\)O and buffer + CaCl\(_2\) is striking. Both remained rigid. Respiration rate was linear throughout, though about 15% lower in the latter. Obviously MgCl\(_2\) was much less effective in countering the action of the buffer. Other experiments indicate that this salt produces erratic results as contrasted with the consistent results obtained with CaCl\(_2\). The decline in the stimulated respiration rate in the buffer coincides with a marked loss in rigidity.

An analysis of variance for these data showed that for flaccidity the effects of various treatments were highly significant (P = 0.01) and the interaction of treatments with hours of treatment was very highly significant (P < 0.01). For respiration the effects of treatments were very highly significant (P < 0.01). There was, however, an appreciable variation from experiment to experiment (different tubers were involved). The interactions of experiments with length of treatment and with kind of treatment were very highly significant (P < 0.01).

The influence of sucrose solutions upon weight changes and flaccidity were affected only slightly by temperatures in the physiological range, as expected (table V). The moment of flaccidity increased promptly in these solutions. From 1 hour onward the tabulated values for MF, though they appear quite variable, actually represent deflections ranging on an average from 1.80 to 1.88 cm, which is less than the interval of observation. The average MF from 1 hour onward in 0.4 M sucrose at 20° is 170 ± 23.9 and at 30° 171 ± 17.5. The lag observed for one-half hour at the lower temperature suggests that this response is in some measure temperature dependent in the more concentrated solution.

On the other hand, temperature has a striking effect upon the action of citric acid (table VI). In terms of total weight loss in the buffer over the 3-hour period the Q\(_{10}\) is about 3. In terms of moment
of flaccidity over the same period the Q10 is in the excess of 500. The Q10 calculated on a rate basis, that is, on the length of time required for the higher temperatures to reach the MF observed after 3 hours at the lower temperature, is about 2.4. Similarly the Q10 for loss in weight in the buffer calculated on a rate basis is about 2.4. These data suggest that during its early stages the loss of rigidity in citric acid has energetics similar to ordinary chemical reactions, but as the process proceeds the apparent energy of activation increases markedly.

Data cited above suggest that at pH 5.5 the citrate buffer had little or no effect. Apparently this is actually an artifact of the conditions of measurement. The above observations were made routinely over a 3-hour period, or less. A series of slices left for 6 hours at room temperature (26°) in 0.005 M citrate at pH 5.5 became very flaccid, whereas controls in water remained turgid. Apparently at the higher pH's the action of citrate is merely slower.

Treatments such as those described above which produced dramatic changes in flaccidity produced no significant changes in the breaking strength of the tissues using the method of measurement described by McClendon and Somers (13). This parameter was measured at the same pH as used in the experiments. With an increase to pH 8 following treatment at a low pH citric acid does lower the breaking strength (13).

Discussion

This method provides a simple means of measuring the rigidity of a slice of tissue and the elastic cantilever beam model appears to be a useful one for interpreting the results. A more elaborate method, useful with small changes in rigidity for which the present one is inadequate, has been provided by Falk, et al. (6) and Nilsson, et al. (15) (see also 5). From their work it appears that the theory of elasticity can be applied to potato tuber tissue. Even the more concentrated citrate buffer was too dilute to produce the changes in weight and rigidity solely as a result of osmosis. Obviously another mechanism is involved. The pH changes suggest an exchange of ions between the cells and the buffer.

It has been suggested that calcium plays an essential role in the structure of plant cell walls (10, 16, 19) and influences their elasticity and/or plasticity (2, 16, 22) by forming a complex with the pectin which is present (1). This latter point of view has been questioned, however (4, 19).

Citrate-phosphate buffers, 0.015 M, pH 4 to 5.5, are particularly effective in extracting calcium from cell walls (3). CaCl2 prevents cell wall breakdown.

Table V. Influence of Temperature upon the Action of Sucrose Solution upon the Flaccidity and Weight of Potato Slices

<table>
<thead>
<tr>
<th>Time hr</th>
<th>Moment of flaccidity g⁻¹ cm⁻²</th>
<th>Loss in wt mg/slice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30°</td>
<td>0.2 M</td>
</tr>
<tr>
<td>0</td>
<td>0.12</td>
<td>0.21</td>
</tr>
<tr>
<td>0.5</td>
<td>0.28</td>
<td>1.67</td>
</tr>
<tr>
<td>1</td>
<td>0.32</td>
<td>1.40</td>
</tr>
<tr>
<td>1.5</td>
<td>0.35</td>
<td>1.84</td>
</tr>
<tr>
<td>2</td>
<td>0.32</td>
<td>2.49</td>
</tr>
<tr>
<td>2.5</td>
<td>0.37</td>
<td>1.51</td>
</tr>
<tr>
<td>3</td>
<td>0.26</td>
<td>1.32</td>
</tr>
</tbody>
</table>

* Initial weight. Each datum is average for 5 slices.

Table VI. Influence of Temperature upon the Action of pH 3.5 Citric Acid-NaOH Buffer upon the Flaccidity and Weight of Potato Slices

The concentration of citric acid was 0.005 M. * Values were estimated from observed deflections.

<table>
<thead>
<tr>
<th>Time hr</th>
<th>Moment of flaccidity g⁻¹ cm⁻²</th>
<th>Loss in wt mg/slice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30°</td>
<td>Buffer</td>
</tr>
<tr>
<td>0</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>0.5</td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>1</td>
<td>0.16</td>
<td>0.52</td>
</tr>
<tr>
<td>1.5</td>
<td>0.15</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>17</td>
</tr>
<tr>
<td>2.5</td>
<td>0.37</td>
<td>820</td>
</tr>
<tr>
<td>3</td>
<td>0.53</td>
<td>560</td>
</tr>
</tbody>
</table>

* Initial weight ± SE; each value represents the averages of 2 experiments each with 5 slices per treatment.
in canned potatoes (17), an effect observed commonly with other canned vegetables. Oxalic acid, also effective in removing calcium, influences the elastic and plastic properties of the Avena coleoptile (12).

It is tempting to suggest that citric acid has its effect upon potato tuber slices because it complexes with calcium in the cell walls and/or elsewhere. This notion is supported by the low concentration of Ca++ which inhibits its action. Citric acid reduces the breaking strength of potato slices when measured at pH 8 (13). This suggests an action upon the cell walls. That the effect of citrate cannot be explained solely, if at all, by chelation of calcium in the cell walls is shown, however, by the observed losses in fresh weight. If only the cell walls were affected, there would be no reason to expect such a loss in weight. Undoubtedly the protoplasm is affected also. The respiration data suggest a delayed action upon the enzyme systems of the cell. Moreover, the action of succinic acid is not to be explained in terms of calcium chelation, unless one assumes that this acid is converted to citric acid via the Krebs cycle. In any case, the results with phosphate buffer show quite clearly that pH external to the tissues is by itself insufficient to explain the results.

Another puzzling aspect of the action of citrate is the fact that the concentrations used are of the same order of magnitude as those reported for the citric acid content in normal tubers (18). Admittedly the low pH and the relatively large volume of buffer used may have overwhelmed the buffering capacity of the tissue slices. However, this seems less likely with citrate buffer at pH 5.5, which also produced a loss of rigidity with longer treatment.

There were changes also in the dimensions of the slices, as indicated by changes in length, but, considering the slice as a homogenous elastic beam, they were inadequate to account for the observed changes in rigidity. Moreover, it seems unlikely that, in view of the cellular structure of these slices, the changes in dimensions of the whole slice have the same meaning as for a solid beam of homogeneous material.

Summary

Slices of Russet Burbank potato tubers were held in a horizontal position by clamping at one end. The downward deflection of the free end was measured and interpreted in terms of rigidity of uniformly loaded, elastic cantilever beams. It was found that citrate caused a rapid loss of rigidity and fresh weight (mostly 2 to 3 hour observation periods). This was particularly marked at pH 3.5 to 4.0. Concentrations of 0.005 and 0.05 M had the same effect. Rigidity and fresh weight decreased much more rapidly at pH 3.5 to 4.0, than at higher values. Calcium ions (0.005 M) effectively inhibited the action of citrate. Magnesium ions gave a lower, erratic inhibition. The action of citric acid was much more pronounced at 30° than at 20°. This acid, at the low pH, stimulated respiration at first but then depressed oxygen uptake over the period when the tissues became flaccid.

That pH external to the tissues was not responsible, by itself, for these responses is indicated by the fact that phosphate buffers at comparable pH's were without effect. Succinic acid at the same pH and concentration was about as effective as citric acid.

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Literature Cited

Iron Stress as Related to the Iron and Citrate Occurring in Stem Exudate

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Iron translocated in stem exudate and separated by electrophoresis moves toward the anode (14). The electrophoretic movement of iron as an anion suggests that iron is combined with some other compound. Tiffin (13) has indicated that organic acids may function directly in the translocation of iron in plants and has identified citrate in the stem exudate by the pentabromoaetone method (6).

The occurrence of citrate and iron in the exudate involves citrate in problems of iron nutrition. Citrate has been associated with such problems for many years. Iljin (7, 8) found that chlorotic plants usually contained more citric and malic acids than normal green plants. Rhodes and Wallace (10) and De-Kock and Morrison (5) have confirmed these findings.

In order to determine a possible role for citrate in the translocation of iron, a comparative study was made of the relationship between iron and citrate in 2 soybean varieties that differ in their ability to absorb and translocate iron (1). PI-54619-5-1 soybean (PI) requires more iron in solution cultures to prevent iron deficiency than does Hawkeye soybean (HA). Reciprocal grafting has shown that the rootstocks are responsible for this apparent difference in iron requirements (4) and provided the basis for making this comparative study.

Methods and Materials

PI-54619-5-1 (PI) and Hawkeye (HA) soybeans were germinated and then grown for 3 days in a modified Steinberg’s solution (12) as previously described (3). Twelve seedlings were then grouped together and precultured in separate jars containing the nutrient solution plus a variable iron treatment where specified. Iron was varied in the nutrient solution from no iron to \( 10 \times 10^{-4} \text{ M} \) iron added as the metal chelate of ethylenediamine-di-(o-hydroxyphenylacetate) (EDDHA). All plants were precultured for 18 days.

After preculture, the plants were transferred to another nutrient solution, called in this paper the absorption nutrient to distinguish it from previous treatments. The absorption nutrient was prepared by adding EDDHA to the nutrient solution (3) and then adding Fe 59 and Fe as FeCl\(_3\) where specified. Immediately after placing the plants in the absorption nutrient, the plant tops were cut off just below the cotyledonary node and stem exudate was collected.

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1 Received August 27, 1964.

*References*


