RESPIRATORY INCREASE AND PHOSPHORUS AND NITROGEN METABOLISM IN SWEET POTATO INFECTED WITH CERATOSTOMELLA FIMBRIATA*

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Many interesting metabolic changes occur in higher plant tissues when they are infected with pathogens. Among them an increase in respiratory rate is one of the most characteristic phenomena, and several suggestions have been put forward to explain this effect. P. J. Allen (1) studied it from the standpoint of phosphate metabolism and observed that in wheat infected with *Erysiphe graminis* there occurred an increase in inorganic phosphate (Pi). He inferred that the respiratory increase may be due to the toxins of the pathogen or the substances produced** in the infected host which might act as uncouplers and accelerate the release of Pi and the accompanied regeneration of adenosine diphosphate (ADP), thus resulting in an increased level of the phosphate acceptor.*** Allen's interpretation was developed by A. Millerd and K. Scott recently (Australian J Biol. Sci., 9, 37 (1955)).

During studies on the mechanism of respiratory increase of sweet potato tissue infected with *Ceratostomella fimbriata* (C.f.), we reported that uncouplers such as ipomeamaron accumulated in the infected part of this plant and stimulated the respiratory rate of the sound tissue (5). However, we have obtained evidence that the natural uncouplers exert potent inhibitory action on the pathogen itself (6, 7), and may in fact play a part in the resisting action of the host plant. Therefore, we deemed it necessary to investigate the respiratory increase in relation to the question whether the uncoupling effect above-mentioned is

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* Part 20 of Phytopathological Chemistry of Black Rot Sweet Potato.
** Personal communication.
*** Allen's interpretation was developed by A. Millerd and K. Scott recently (Australian J Biol. Sci., 9, 37 (1955)).
auxiliary in its nature, and whether there might be any other explanation for the stimulation observed.

In an attempt to answer these questions, a series of experiments has been carried out in which changes in phosphorus and nitrogen compounds were measured together with the respiration and its response to 2,4-dinitrophenol (DNP).

EXPERIMENTAL

Sweet potato (variety, Norin No. 1) was sliced into several pieces of 2–3 mm. thick, and a spore suspension of C.f. was sown on the surface of these slices, which were then incubated at 25°.

Uninoculated slices were served as control test for the effect of slicing under the same conditions. Under the present conditions, the fungus grew rapidly and, after about 48 hours, it spread over the surface and also penetrated into the sweet potato tissue; thereafter the growth declined gradually. In the present study, slices (2–3 mm. thick) from non-infected region (as determined microscopically) next to the infected (0.2–0.5 mm. thick) was taken to be assayed for various compounds concerned.

Respiratory Experiment

Small slices were taken from sound tissue, and the oxygen uptake was determined by the manometric technique. Experimental methods are shown in the legend of Table I. The DNP effect was determined by the respiratory increase caused by its addition in a final concentration of $5 \times 10^{-5} M$. DNP was most effective at this concentration both in the infected sweet potato and in the control.

Each value represents the average of duplicate determinations.

Fractionation

The method of Ogur and Rosen (8), was employed with slight modifications as shown schematically in Diagram 1. Phosphate was determined by Nakamura’s method (9), and nitorogen by the micro-Kejldahl method. Ribonucleic acid (RNA) was estimated by absorption at 260 mμ by Shimadzu quartz spectro-photometer. Protein nitrogen was analysed by Howe’s method (10).

RESULTS

1. Changes in the rate of respiration after inoculation with C.f., and the enhancement by DNP are shown in Table I. In contrast to the nearly constant rate of respiration of the control with and without addition of DNP, the increase in respiration of the infected sweet potato reached a maximum at 48–72 hours, and decreased thereafter. The appropriate increase (80 per cent) caused by DNP dropped to about 30 per cent in the period of 48–72 hours, but recovered to the same level as
that of the control at the later stage (168 hours). Since the respiratory increase brought about by DNP is dependent on the acceleration of the reaction $\text{ATP} \rightarrow \text{ADP} + \text{Pi}$ in such a tissue, the values for the effect may indicate the speed of the above reaction in the tissues. It appears that the smaller DNP effect in the infected sweet potato may be caused by the acceleration of the reaction $\text{ATP} \rightarrow \text{ADP} + \text{Pi}$ in such tissue. This might occur by the promotion of breakdown of ATP and/or the utilization of ATP by some synthetic reaction. Indeed, from our data on the increase in the activity of respiratory enzymes such as cytochrome oxidase and polyphenol oxidase in the infected sweet potato tissue (II),

**Diagram 1**

*Fractionation Method for Phosphorus and Nitrogen Compounds in the Infected Sweet Potato*

1. Infected sweet potato
2. Remove the infected part
3. 5 g. of the sound tissue and 25 ml. of 20% $\text{C}_2\text{H}_5\text{OH}$ containing 12% $\text{HClO}_4$
4. Homogenize, 5 min. at $0^\circ$
5. 5 ml. of homogenate
6. Centrifuge for 10 min. at 3000 r.p.m.
7. Wash the residue with 5 ml. of 20% $\text{C}_2\text{H}_5\text{OH}$ containing 12% $\text{HClO}_4$
8. Centrifuge again at 3000 r.p.m.

**Supernatant**

- Extract with 5 ml. of alcohol-ether (3:1)
- Extract again with 5 ml. of alcohol ether (3:1)

**Residue**

- Extract with 5 ml. of $N\text{ HClO}_4$
- Overnight at $0^\circ$
- Wash again

**Supernatant**

- Phospholipide $\text{P}$

**Residue**

- RNA-P
  - 260 m$\mu$
- Protein $\text{N}$
  - $\text{H}_2\text{SO}_4$ digestion
TABLE I

The Respiration of Infected and Sound Sweet Potato and the DNP Effect

<table>
<thead>
<tr>
<th>Time after infection</th>
<th>hrs.</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>µl.</td>
<td>77.3</td>
<td>88.0</td>
<td>80.5</td>
<td>77.6</td>
<td>93.7</td>
</tr>
<tr>
<td>Control DNP</td>
<td>µl.</td>
<td>138.7</td>
<td>156.0</td>
<td>143.0</td>
<td>149.0</td>
<td>178.0</td>
</tr>
<tr>
<td>Increase</td>
<td>%</td>
<td>80</td>
<td>77</td>
<td>78</td>
<td>92</td>
<td>90</td>
</tr>
<tr>
<td>Infected</td>
<td>µl.</td>
<td>132.7</td>
<td>147.0</td>
<td>177.0</td>
<td>120.0</td>
<td></td>
</tr>
<tr>
<td>Infected DNP</td>
<td>µl.</td>
<td>208.0</td>
<td>186.0</td>
<td>232.0</td>
<td>231.0</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>%</td>
<td>56</td>
<td>26</td>
<td>31</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

Twenty slices (7 mm. in diameter and 0.5 mm. thick) were put into Warburg vessels containing 85 µM of phosphate buffer (pH 5.5) and 162 µM of sucrose in total volume of 2 ml. Gas phase, air; temp., 30°; 20 min. equilibration; DNP was added in a final concentration of 5×10⁻⁵ M.

it is natural to suppose that the enhancement of ATP-utilizing reactions such as synthesis of enzyme proteins causes a concomitant respiratory increase. To clarify this point, the tissues were submitted to fractional analyses for several phosphorus compounds.

2. Representative data for phosphorus compounds are shown in Fig. 1. While both Pₐ and organic phosphate (Pₒ) of the control remained nearly constant with time, Pₐ in the sound part of the infected sweet potato decreased at the beginning, increased in the second stage, and dropped again, with concomitant increase in Pₒ. A constant Pₐ level in the secondary stage was observed occasionally. There was no significant difference between the infected sweet potato and the control tissue in their alcohol-ether soluble fraction containing phospholipide and in the cold HClO₄-extractable fraction containing RNA (Table II). The Pₒ formation in the sound tissue next to the infected tissue may thus indicate the activation of a phosphate cycle by which the energy of ATP is utilized in some way, resulting in the increase of the rate of ADP generation. But the observed increase in Pₐ at 24–48 hours and the minor effect of DNP on respiration may show the partial participation of ipomeamarin in the uncoupling effect or/and activation of adenosine triphosphatase (ATP-ase) which may be related to protoplasmic streaming and changes in permeability.

3. From the quantitative analysis of the phosphates, it was
presumed that the anabolism of sweet potato tissue might be enhanced when it was infected with the pathogen. To elucidate this phenomenon more thoroughly, our attention was directed to the nitrogen metabolism, in particular, to the pattern of protein synthesis in the tissue. As shown in Fig. 2, the decrease in nitrogen compounds in the acid soluble fraction (amino acids and amides) is compensated by the concomitant increase in acid insoluble nitrogen compounds (proteins). The observed increase in proteins is one of the most typical anabolic phenomena of the infected plant. In Table III are shown the results of analyses for protein by Howe's method which has been applied to the analysis of serum protein. These data are also in accord with the above results.

**DISCUSSION**

French and Beevers have suggested that the increased respiration brought about when auxins are added to plant tissue may be due to the activation by the hormone of a system utilizing ATP (12). The DNP effect on the respiration of such an auxin-treated plant tissue is known to be less than that of the control (13). It has been shown by Pearson and Robertson (14) that the climacteric rise of apple in ripening process is due to the synthesis of respiratory enzyme proteins leading to the useful breakdown of ATP. However, Millerd et al.
(15) explained the mechanism of climacteric rise of avocado ripening as the acceleration of breakdown of ATP by ATP-ase or by some uncouplers. Our results represent an additional example of a similar regulatory mechanism in plant respiration. Synthetic events in the host are apparently stimulated by the infection of pathogens, and the resulting more rapid turnover of ATP is thought to bring about the respiratory increase observed. The uncoupling effect of metabolites such as ipomeamaron seems to be of relatively minor importance.

There is an increasing accumulation of many metabolites in the sound tissue adjacent to the infected tissue in parallel with the increase in respiration, and most of them may be concerned in some way with the resistance of the sweet potato to the pathogens (16). Besides metabolites of lower molecular weight thermolabile and non-dialyzable fraction having resisting action is produced in the sound part of the infected sweet potato. Possibly the resisting components may be the specific proteins such as the antiinflammatory factor of Menkin (17),

<table>
<thead>
<tr>
<th>Time after infection, hrs.</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh sweet potato</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>95</td>
<td>94</td>
<td>100</td>
<td>94</td>
<td>95</td>
</tr>
<tr>
<td>Infected</td>
<td>99</td>
<td>98</td>
<td>90</td>
<td>90</td>
<td>98</td>
</tr>
</tbody>
</table>

Phospholipide-P was analyzed by estimation of inorganic phosphate after digestion of alcohol-ether soluble fraction with HClO₄.*

<table>
<thead>
<tr>
<th>Time after infection, hrs.</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh sweet potato</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>48.8</td>
<td>50.0</td>
<td>48.7</td>
<td>42.5</td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>53.0</td>
<td>51.5</td>
<td>47.4</td>
<td>44.6</td>
<td></td>
</tr>
</tbody>
</table>

RNA-P was estimated by the ultraviolet absorption at 260 mμ of cold N-HClO₄ extracted fraction*.

* Expressed as γ/g. fresh tissue.
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Fig. 2. Change of the amounts of nitrogen fraction in the infected sweet potato.

$N_{\text{sol.}}$ in the figure is expressed as the difference of the acid-soluble nitrogen fraction in the control from that in the infected sweet potato, and $N_{\text{insol.}}$ as the difference of acid-insoluble nitrogen fraction in the infected sweet potato from that in the control. The values of the control were scarcely changed throughout the experimental stage.

properdin system, a factor of natural immunity (18), or certain enzymes which may damage the pathogen or break down the poisonous metabolites of it. As the another way in which the protein may participate in the resistance, the following assumption is possible. The protein in the sound part adjacent to the infected is unstable as well as active; when the cells of the sound part are affected by the pathogen, the protein may be denatured rapidly, interact with the accumulated polyphenols, thus form the barrier against penetration of $C. f$. The rise in anabolic process observed in infected sweet potato is the unique phenomenon of infected plant tissues, and it is important to discuss this finding in relation to host resistance. Tomiyama et al. (19) reported the similar phenomenon in the white potato infected with Phytophthora.
Three days after the inoculation of C. f. the samples were prepared in the way shown in the Experimental. The amounts of crude protein in the samples were estimated by micro-Kjeldahl method, after $\text{H}_2\text{SO}_4$ digestion. The samples were homogenized with equal amounts of cold water and centrifuged, the supernatants were fractionated by the method of Howe. Each fraction was estimated by micro-Kjeldahl method, and the N amount was multiplied by 6.25, and expressed as the amount per 100 g. of tissue (wet weight).

TABLE III
*The Distribution of Nitrogen-Containing Substances in Infected and Sound Sweet Potato*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Infected</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>69.4 %</td>
<td>67.2 %</td>
</tr>
<tr>
<td>Crude protein</td>
<td>1.04</td>
<td>1.06</td>
</tr>
<tr>
<td>Juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>989 mg.</td>
<td>1046 mg.</td>
</tr>
<tr>
<td>$\text{Na}_2\text{SO}_4$ 14 g./10 ml.</td>
<td>742</td>
<td>568</td>
</tr>
<tr>
<td>$\text{Na}_2\text{SO}_4$ 18 g./10 ml.</td>
<td>19</td>
<td>32.2</td>
</tr>
<tr>
<td>$\text{Na}_2\text{SO}_4$ 22 g./10 ml.</td>
<td>74.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Unprecipitable fraction</td>
<td>140.6</td>
<td>360.9</td>
</tr>
</tbody>
</table>

Three days after the inoculation of C. f., the samples were prepared in the way shown in the Experimental. The amounts of crude protein in the samples were estimated by micro-Kjeldahl method, after $\text{H}_2\text{SO}_4$ digestion. The samples were homogenized with equal amounts of cold water and centrifuged, the supernatants were fractionated by the method of Howe. Each fraction was estimated by micro-Kjeldahl method, and the N amount was multiplied by 6.25, and expressed as the amount per 100 g. of tissue (wet weight).

**SUMMARY**

1. Changes in the metabolism of sweet potato tissue induced by infection with *Ceratostomella fimbriata* were studied.

2. The respiration of sound tissue adjacent to the infected tissue was about twice greater as much as the control 72 hours after the fungus inoculation; thereafter it dropped. Rate of the respiratory increase due to DNP addition was reciprocal to that of the respiratory increase caused by the fungus infection, and the finding was assumed to be caused by the acceleration of ADP generation in the tissue.

3. In the sound tissue next to the infected, inorganic phosphate decreased in line with organic phosphate formation, and a concomitant increase of acid insoluble nitrogen compounds (protein) occurred, a decrease of acid soluble nitrogen compounds (amino acids and amides) being also observed. These facts indicate an activation of anabolism...
in the infected sweet potato, but occasionally at one stage (72 hours) 
Pi increase was found, and the partial participation of an uncoupling 
reaction or the activation of ATP-ase is suggested.

4. In the infected sweet potato, the levels of phospholipide and 
RNA remained unchanged.

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