EFFECTS OF VITAVAX ON CHLOROPHYLL CONTENT, PHOTOSYNTHESIS AND RESPIRATION OF BARLEY LEAVES

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ABSTRACT
Detached barley leaves treated with the systemic fungicide, 2,3-dihydro-5-carboxanilido-6-methyl-1,4,oxathiin (Vitavax), remained green longer than nontreated leaves. The chlorophyll content of treated leaves was slightly higher than that of nontreated ones during the first 7 days, and markedly higher 20 days after treatment. The immediate effect of the chemical was inhibition of photosynthesis and respiration. Treated leaves returned to apparently normal photosynthesis and respiration within 5 days; however, their mg dry weight/g fresh weight ratio remained lower than that of nontreated leaves, even 20 days after treatment.

INTRODUCTION
The effects of fungicides on photosynthesis and respiration in treated plants have been reported by several workers (2, 3, 5, 8). All petroleum sprays applied to citrus leaves inhibited photosynthesis, while some inhibited or stimulated respiration depending on the type of hydrocarbon oil used (5). Sodium dimethyldithiocarbamate (Na-DMDT) and tetramethylthiuram disulfide (TMTD) inhibited photosynthesis, and did not affect endogenous respiration in Enteromorpha linza (L.) J. Ag. (3). Other chemicals such as dihydrostreptomycin, cycloheximide (Actidione) and dichloroanalinotriazine (Dyrene) influence chlorophyll production (7, 8).

The chemical 2,3-dihydro-5-carboxanilido-6-methyl-1,4,oxathiin (Vitavax) has been found effective in controlling barley loose smut [Ustilago nuda (Jens.) Rostr.] (6). The present study was done to determine the effect of Vitavax on the physiology of the barley plant, in order to aid in understanding its mode of action. Chlorophyll content, photosynthesis and respiration in barley leaves treated with Vitavax were studied.

MATERIALS AND METHODS
Leaves from young barley plants, cultivar Larker, in the 3- to 4-leaf stage were used as the source of plant material. The chemical 2,3-dihydro-5-carboxanilido-6-methyl-1,4,oxathiin (Vitavax), courtesy of Uniroyal Chemical, Division of Uniroyal, Inc., was sprayed on the leaves at a rate of 3.6 g/liter of water, with 5 ml Tween 80 added to ensure uniform leaf coverage. The control plants were sprayed with a comparable Tween 80 solution.

Samples were collected at random from treated and nontreated barley plants. Only the second foliar leaf was detached and used in all experiments. Tentcentimeter sections of five leaves from each treatment, weighing from 500 to 900 mg depending on the stage of growth, were used. The turgidity of the leaves was maintained by inserting the cut ends into slits in the side of a small water-filled plastic vial. Both treated and nontreated samples were placed in Plexiglas chambers, which were submerged in a water bath held at 20 ± 1 C. The chambers were continuously flushed with outside air containing about 380 ppm CO₂ and tempered to 20 C. The rate of air flow was approximately 1 liter/min.

Photosynthesis and respiration measurements were made by infrared gas analysis using a Beckman IR15A with an attached microvolt recorder, following the
Table 1. Dry weight and chlorophyll content of Vitavax-treated and nontreated barley leaves

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>Mg dry wt/g fresh wt</th>
<th>Dry wt mg/cm²</th>
<th>Mg chlo./g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T*</td>
<td>NT*</td>
<td>NT/T</td>
</tr>
<tr>
<td>1</td>
<td>125</td>
<td>146</td>
<td>1.17</td>
</tr>
<tr>
<td>3</td>
<td>129</td>
<td>144</td>
<td>1.12</td>
</tr>
<tr>
<td>5</td>
<td>136</td>
<td>152</td>
<td>1.12</td>
</tr>
<tr>
<td>7</td>
<td>140</td>
<td>154</td>
<td>1.10</td>
</tr>
<tr>
<td>20</td>
<td>136</td>
<td>162</td>
<td>1.19</td>
</tr>
</tbody>
</table>

*T = treated leaves, NT = nontreated leaves.

Table 2. Carbon dioxide uptake by Vitavax-treated and nontreated barley leaves during photosynthesis, measured at 20-min intervals beginning 25 min after treatment

<table>
<thead>
<tr>
<th>Time after treatment (minutes)</th>
<th>T</th>
<th>NT</th>
<th>NT/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.13</td>
<td>0.55</td>
<td>4.17</td>
</tr>
<tr>
<td>45</td>
<td>0.08</td>
<td>0.62</td>
<td>7.37</td>
</tr>
<tr>
<td>65</td>
<td>0.05</td>
<td>0.62</td>
<td>12.00</td>
</tr>
<tr>
<td>85</td>
<td>0.03</td>
<td>0.63</td>
<td>20.29</td>
</tr>
<tr>
<td>105</td>
<td>0.03</td>
<td>0.63</td>
<td>21.62</td>
</tr>
<tr>
<td>125</td>
<td>0.03</td>
<td>0.62</td>
<td>23.85</td>
</tr>
<tr>
<td>145</td>
<td>0.01</td>
<td>0.62</td>
<td>44.43</td>
</tr>
<tr>
<td>165</td>
<td>0.01</td>
<td>0.61</td>
<td>50.75</td>
</tr>
</tbody>
</table>

Table 3. Carbon dioxide uptake by Vitavax-treated and nontreated barley leaves during photosynthesis, measured every other day beginning one day after treatment

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>T</th>
<th>NT</th>
<th>NT/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.12</td>
<td>0.68</td>
<td>5.77</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>0.74</td>
<td>1.50</td>
</tr>
<tr>
<td>5</td>
<td>0.54</td>
<td>0.59</td>
<td>1.09</td>
</tr>
<tr>
<td>7</td>
<td>0.52</td>
<td>0.54</td>
<td>1.03</td>
</tr>
<tr>
<td>20</td>
<td>0.41</td>
<td>0.39</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Table 4. Carbon dioxide given off by Vitavax-treated and nontreated barley leaves during respiration measured every other day beginning one day after treatment

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>T</th>
<th>NT</th>
<th>NT/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.039</td>
<td>0.070</td>
<td>1.79</td>
</tr>
<tr>
<td>3</td>
<td>0.048</td>
<td>0.054</td>
<td>1.13</td>
</tr>
<tr>
<td>5</td>
<td>0.043</td>
<td>0.047</td>
<td>1.09</td>
</tr>
<tr>
<td>7</td>
<td>0.047</td>
<td>0.052</td>
<td>1.11</td>
</tr>
<tr>
<td>20</td>
<td>0.031</td>
<td>0.035</td>
<td>1.13</td>
</tr>
</tbody>
</table>
general procedure as outlined by Jensen (1). Incandescent lamps were used to
illuminate leaves with 19,400 lux during photosynthesis. Respiration measure-
ments were made in complete darkness.

Chlorophyll content, photosynthesis and respiration of barley leaves were
measured every other day, starting 1 day after treatment and continuing for
7 days. Final measurements of photosynthesis and respiration were made 20 days
after treatment. Detached leaves were also sprayed and photosynthesis was
measured at 20-min intervals starting 25 min after treatment and continuing for
165 min.

Dry weight and chlorophyll samples were taken from the material used in
each experiment. One-half of the sample was dried at 60°C for 24 hr for dry
weight; the other half was frozen and used for chlorophyll extraction. Chlorophyll
was extracted with 80% acetone and measurements were made at 645 μm and
663 μm with a Beckman DU spectrophotometer.

Leaf areas were measured immediately after each experiment, using photo-
sensitive blueprint paper. The leaf area on the paper was cut out, weighed and
converted to cm².

RESULTS

Dry weight, expressed as mg dry weight/g fresh weight and mg dry weight/cm²
was less in treated than in nontreated leaves. This difference remained relatively
constant throughout the experiment (Table 1). Chlorophyll content in treated
leaves was higher than in nontreated leaves. The ratio of nontreated to treated
1 day after treatment was 0.9, and after 20 days 0.78 (Table 1).

Photosynthesis was inhibited within 25 min after treatment (Table 2). The
nontreated-to-treated ratios 25 min and 165 min after treatment were 4.17 and
50.75, respectively.

Inhibition of photosynthesis was still apparent 1 day after treatment (Table 3).
Nontreated leaves took up over five times as much CO₂ as did treated leaves.
Five days after treatment, the difference in μl CO₂ uptake/min per cm² between
treated and nontreated leaves was small, indicating photosynthetic recovery.
Twenty days after treatment, photosynthetic rate expressed as μl CO₂/min per cm²
was slightly greater in treated than in nontreated leaves.

Respiration also was inhibited, but to a lesser degree (Table 4). The ratio
of nontreated to treated was 1.56 after 1 day and 1.00 after 7 days. Recovery of
normal respiration was apparent 3 days after treatment.

DISCUSSION

The immediate effect of Vitavax was almost complete inhibition of photosynthesis.
Similar effects on photosynthesis have been reported for other fungicide chemicals
(2, 3, 5). The physiological mechanism was not evident from the study. A non-
specific action of the chemical is suggested from data on inhibition of respiration.

Inhibition of photosynthesis by hydrocarbon oils is thought to be a mechanical
interference of gaseous exchange (5). Other chemicals actually enter the cells,
for instance, TMTD and Na-DMDT, and interrupt the photosynthetic process
(3). Studies on the uptake and binding of Vitavax by Ustilago maydis (D.C.)
Cda and Rhizoctonia solani Kuhn have shown the chemical to be bound in the
ribosomal-soluble fraction of the cells (4). While no attempt was made during
this study to determine the actual uptake of Vitavax, it could be postulated that
similar uptake and binding occur in leaf cells of the barley plant. The fungicide, once in the cell, may be bound on the chloroplasts, thus interfering with photosynthesis.

Recovery of photosynthesis in treated barley plants starts within 24 hr and is complete after 72 hr. This phenomenon could not be explained by the present study.

Treated leaves contained more water than nontreated leaves, even 20 days after treatment, suggesting that Vitavax had an effect on selective permeability of the cells and its effect was prolonged. Mathre (4) also suggests that an alteration in selective permeability occurs in treated fungal cells.

Vitavax-treated barley leaves stayed green longer than nontreated leaves, indicating that chlorophyll disappeared more rapidly in nontreated than in treated leaves. This was borne out in studies on the chlorophyll content of treated and nontreated barley leaves, where the chlorophyll content ratio of nontreated to treated decreased from 0.96 to 0.78 over a 20-day period.

REFERENCES