Metabolism of Indoleacetic Acid in Rust Diseases. I. Factors Influencing Rates of Decarboxylation

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Introduction

One of the results of the infection of a higher plant by a pathogen may be a change in the rates of metabolism of hormones. It is possible that hormone concentrations are of importance in controlling the development of both host and pathogen. Most obviously, abnormal growth of the host may result from changes in hormones caused by the prolonged development of the pathogen in the host tissue. In such instances, the role of hormones essentially is a side effect of no consequence to parasite establishment. However, it is conceivable that the initial establishment of the pathogen may depend upon hormonal changes caused by the entry of the pathogen. In this second case, the changes may be confined to the few cells at the site of infection or may be too transient to cause malformations, even if the plant organ is competent to respond by growing.

Some previous work (14, 16, 23, 24, 34) has shown that higher levels of IAA occur in several plant species infected by rust fungi which cause distinct growth disturbances. Usually the data were obtained in late stages of infection when the parasite was in a phase of spore production rather than vegetative growth. Studies (4, 6) of infection by Puccinia carthami on hypocotyls of safflower suggested correlations among mycelial growth and disease-induced respiration, host growth and quantitative increases in auxin levels. The increase in auxin could be detected in the very early stages of disease. It was postulated that primary contact by the fungus in susceptible tissue triggered an increase in hormones which induced host changes conducive to continued growth of the parasite. It was suggested that factors, possibly metabolic, which are controlled by hormones were common to vegetative growth of both host and parasite. Although it was suggested (31) that IAA might modify pathways of metabolism, subsequent work (5) indicated that the low C4/C1 ratios characteristic of rust-affected tissue under controlled conditions is not induced by IAA.

Extension of these ideas to cereal rusts such as stem rust of wheat is difficult because visual growth disturbances are absent. Certain treatments (17, 27, 31) which may be related to hormonal changes can cause breakdown in resistance of wheat to rust. The data available (29) indicate higher IAA levels in diseased plants but consist of one analysis late in disease development when spore production was well under way. Shaw and Hawkins (29) did find that infected tissue differed from normal leaves in capacity to decarboxylate exogenous IAA. In agreement with Pilet (23, 24), they observed that IAA degradation was lower in diseased tissue several days after inoculation. In the earlier stages of infection, IAA decarboxylation was much greater than normal. The rapidity with which infected tissue changed from the initially high rates of destruction to the reduced rates appeared to be related to susceptibility or resistance. Susceptible tissue appeared to revert more quickly than resistant tissue. They postulated tentatively that resistance or susceptibility might be determined by the speed with which IAA accumulated due to inhibition of the oxidase.

During a study of fixation of metabolically generated C14O2 by rust-affected wheat leaves, IAA labelled in the carboxyl group was fed to tissues. We observed certain aspects of the kinetics of decarboxylation of IAA which had not been reported previously. Further observations on the factors affecting this reaction suggested that current ideas of the metabolism of IAA in rusted wheat leaves require modification especially with regard to the differences between resistant and susceptible tissue.

Materials and Methods

Growth and Inoculation of Plants. Wheat (Triticum aestivum L. var. Little Club) was planted in growth rooms at 22° under a 13 hour photoperiod. Six or 7 days after planting, the fully expanded primary leaf was dusted lightly with a mixture of talc and uredospores of Puccinia graminis tritici (Erik. and Henn.) race 56. A similar group of plants was dusted lightly with talc only and placed with the inoculated plants in the same dew chamber.

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held at 22°. Plants dusted with talc only are referred to as chamber controls. Plants which were kept continuously in the growth room served as bench controls.

Since experiments performed subsequent to most of those reported here suggested that light regime is important in determining infection and capacity of tissue to decarboxylate IAA, a brief description of lighting is appropriate. Light in the growth rooms was furnished by a bank of cool-white fluorescent bulbs which gave 1400 ft-c at the level of the plants. In addition there was an extraneous source of incandescent light furnished by a 10 w amplex pilot bulb used to signal the flow of refrigerant into the cooling coil. About 9 hours after the start of the light period on the day of inoculation, plants to be tested were transferred for 15 minutes to rooms illuminated with incandescent light. This day is designated as day 0 in subsequent charts and tables. After control and inoculated plants were placed in the incubation chamber, they were given an additional 4 hours of fluorescent light to complete the 13 hour photoperiod. At the start of the photoperiod for the next day (day 1) diseased plants and chamber controls were returned to the original growth rooms and randomized with the bench controls.

In any one experiment, chamber controls and diseased plants received identical light treatments. However, it was not possible, for several reasons, to ensure that equivalent light treatments were obtained from experiment to experiment.

Measurement of IAA Decarboxylation. In the daily analysis of decarboxylation rates, samples were obtained at a constant time interval (usually 1 hour) after the start of the light period. Approximately 40 leaves of tissue from diseased plants, bench controls and chamber controls were cut into 0.5 cm lengths under a mixture of daylight and fluorescent light in the laboratory. After rinsing the samples with distilled water, surface moisture was removed with paper towels. One-half gram of each tissue was weighed into triplicate vessels of the type described previously (5). The vessels contained 5 ml of 0.1 M KH₂PO₄, and 50 μg of IAA labelled in the carboxyl group. They were sealed with rubber stoppers from which was suspended a glass planchet containing a circle of lens paper and 0.1 ml of 10 % KOH to trap CO₂. The vessels were shaken in air in the growth room on a platform shaker covered with several thicknesses of black cloth to exclude light. At intervals (usually 2, 4, and 6 hours) the vessels were exposed to light for 5 to 15 seconds in order to insert fresh planchets.

After drying at 60°, the planchets were counted in gas-flow system and the data converted to percentage decarboxylation. The initial radioactivity in the vessels was determined each day as the average of five 0.1 ml samples obtained at random from separate vessels and dried in planchets containing lens paper and KOH. In some experiments when the amount of radioactivity in tissue was determined corrections were made for self-absorption.

Radioactive IAA was obtained in 3 separate lots from Nichem, Inc. Two preparations were white crystalline materials which migrated as IAA in isopropanol-ammonia-water (10:1:1 (INW) and gave the usual colors with Salkowski and Ehrlich's reagents. The third lot was light-brown crystals which also migrated as a single radioactive peak in INW, butanol-ethanol-water (4:4:1) and butanol-benzyl alcohol-formic acid-water (43:43:5:9). No Salkowski or Ehrlich positive contaminants were observed. Stock solutions were prepared by dissolving 3 to 6 mg in 0.2 ml of 95% ethanol and quickly diluting with 0.1 M KH₂PO₄ to give a final concentration of 50 μg IAA per ml. One ml was added to 4 ml of buffer in the experimental vessels. The final concentration of alcohol during assay was always less than 0.04 %.

The agreement among the 3 replicates employed was not as good as in other studies using the same general procedures. Usually the deviation was less than 10% when the totals for 6 hours were calculated. Occasionally, variations as high as 30% was found in individual samples obtained at intervals during the assay. Some variation probably was due to trapping of CO₂ in clumps of tissue within the vessel. Results from a typical experiment are given in detail in table I in order to show the variation encountered. We do not consider that differences among treatments of less than 20% of rate of decarboxylation are significant unless they were repeated several times. A check of spontaneous decarboxylation by IAA during the absence of tissue showed no more than 0.5% of the original activity appeared in the planchet during 6 hours and this may represent some volatilization of IAA (see table I). Since the amount found at any interval was usually less than 1% of the value obtained with tissue, no corrections for this quantity were made.

Results

Kinetics of IAA Decarboxylation. Shaw and Hawkins (29) reported that 85% of the decarboxylation occurred within the first 2 hours of a 3 hour treatment. Preliminary experiments under our conditions do not agree with this. Age of tissue appears to be an important determinant of the time course of decarboxylation. Figure 1 illustrates 4 comparisons of healthy and diseased first leaves from young (2,3 days) and older (9,10 days) plants. Although it would be possible to draw smooth curves for all data, a close examination of the figures indicates that most frequently the slope for the first 2 hours is less than for subsequent time periods, perhaps because uptake from solution is limiting. Maxi-
in 2 experiments which resulted in development of 2 levels of infection based on counts from 15 leaves is depicted in figure 2. Although both infections appeared visually to be heavy, the second experiment actually had approximately twice the infection intensity. Bench and chamber controls decarboxylated about the same amount of IAA in 6 hour periods. As healthy tissue matured, there was a progressive increase in ability to decarboxylate exogenous IAA; in the second experiment nearly 60% of added IAA was metabolized via decarboxylation on the 11th day after inoculation (approximately 14 days after emergence).

The sequence of events in diseased tissue occurred in 3 stages: A, before fleck symptoms (F), a marked increase over controls; B, during production of flecks and early sporulation, little difference from controls; C, after sporulation (S) was well advanced, a marked depression below controls.

This separation into 3 phases differs from Shaw and Hawkins' recognition of 2 phases. In figures 6, 7, and 8 of reference 29 decarboxylation data are presented as percentage of control tissue at intervals

![Figure 1. Time course of dark decarboxylation for young (lower sections) and older (upper sections) healthy (H) and infected (D) first leaf of wheat. Tissue collected 2, 3, 9, and 10 days after inoculation with Puccinia graminis tritici, race 56. IAA 50 μg per 0.5 g tissue. The curvilinear response of young healthy tissues should be noted.](image)

maximum differences in decarboxylation between healthy and diseased leaves were obtained at 6 hours.

A second aspect of the data is the difference in shape of the curves for young and old healthy tissue. In several experiments the rates of decarboxylation by young tissue decreased with time while older tissue decarboxylated IAA at much more rapid rates which were essentially linear. (See also table III for young tissue.) The data for healthy tissue might be interpreted to mean that enzyme per se is not limiting in young tissue but perhaps that a cofactor is limiting or that products of the reaction become inhibitory. In instances where diseased tissue decarboxylated at different rates than healthy, the rates were linear, never decreasing markedly during the final 2 to 3 hours of assay.

Although it was possible to continue experiments for longer times, it was decided to make comparisons at the end of 6 hours in order to minimize microbial contamination.

Changes in IAA Decarboxylation during Rust Development. Percentage decarboxylation of IAA 50 μg IAA C14OOH by 0.5 g of wheat first leaf tissue. Lower section is data from an infection resulting in 115 pustules per leaf; upper section from experiment with final infection of over 200 pustules per leaf. F denotes fleck symptom; (S) denotes trace of sporulation; S preceded by numeral denotes percentage of pustules producing spores.

![Figure 2. Percentage dark decarboxylation of 50 μg IAA C14OOH by 0.5 g of wheat first leaf tissue. Lower section is data from an infection resulting in 115 pustules per leaf; upper section from experiment with final infection of over 200 pustules per leaf. F denotes fleck symptom; (S) denotes trace of sporulation; S preceded by numeral denotes percentage of pustules producing spores.](image)
after inoculation. The data in figure 7 suggest that in susceptible hosts there is a peak of oxidase activity on the first or second day after inoculation (phase I) followed by a rapid decline so that by the fourth day IAA oxidase activity is appreciably below that of the control tissue (phase II). Resistant hosts respond somewhat later and oxidase activity is not below that of the control tissue until 7 or 8 days after inoculation. It is presumed that IAA levels follow an inverse relationship.

Figures resembling, but not identical to, the idealized curves of Shaw and Hawkins can be obtained by transforming the data of figure 2 for diseased tissue to percentage of chamber control decarboxylation for successive days (fig 3, 4 upper half; each day). The 3 stages described above are clearly apparent.

A limitation of this method of data presentation is the fact that healthy tissue is undergoing a normal, progressive increase in decarboxylation activity. In an attempt to arrive at a better understanding of the relationships between host and parasite development, the same data are plotted as percentage of the control value at day 1 (lower graphs of figure 3, 4). In experiment 1 the last 2 days for control tissue were data obtained from bench, rather than chamber, controls.

There is essentially a linear increase in capacity of healthy tissue to decarboxylate IAA. In contrast, after an initial burst, diseased tissue appears to revert slowly to the low level of activity found in young healthy tissue. With the possible exception of day 10 in experiment 2, the diseased first leaf of wheat did not fall below the activity of normal tissue which had just attained maximal expansion. The marked differences between healthy and diseased tissues after the onset of sporulation are due largely to the increased capacity of healthy older tissue to decarboxylate IAA rather than to pronounced inactivation in diseased tissue of an enzyme which functioned at an initially high rate at the time of inoculation.

Effect of Leachates of Rusted Tissue on Decarboxylation of Healthy Tissue. Pilet (23) obtained evidence for the presence in diseased tissues of materials inhibitory to IAA oxidase in vitro. Further, van Sumere et al (35) observed phenolic compounds in spores and Kiraly and Farkas (18) have observed the synthesis of phenols in rusted tissue. It was necessary to determine if the results after sporulation
Table I

<table>
<thead>
<tr>
<th>Treatment</th>
<th>cpm in replicate</th>
<th>% decarboxylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy tissue</td>
<td>8,600</td>
<td>10.8</td>
</tr>
<tr>
<td>Diseased tissue</td>
<td>4,000</td>
<td>5.0</td>
</tr>
<tr>
<td>Control—no tissue</td>
<td>270</td>
<td>0.4</td>
</tr>
<tr>
<td>Leachates from diseased tissue</td>
<td>960</td>
<td>1.2</td>
</tr>
<tr>
<td>Healthy tissue + leachates from diseased tissue</td>
<td>8,800</td>
<td>11.6</td>
</tr>
</tbody>
</table>

might be due to substances leached from the infected leaves and accompanying spores. Since there were numerous spores present, the washings from diseased tissue were checked for ability to decarboxylate IAA. The results obtained are given in Table I along with appropriate controls.

Obviously, leachates from rusted tissue did not inhibit decarboxylation. Although washings alone accounted for 1.2% decarboxylation of exogenous IAA, this represents the maximum possible contribution of spores in data given for diseased tissue. This stems from the fact that diseased tissue was washed thoroughly before assay, hence most spores present in the washings of this experiment would be absent during experiments such as those of figure 1.

**Fate of Radioactivity in Tissues and Bathing Solutions.** In an effort to define another parameter of this assay, the radioactivity in the bathing solution and the tissue were determined for experiment 1 (fig 2).

Of particular interest in table II are the facts that the radioactivity in the bathing solutions surrounding healthy tissue is the same throughout the experiment irrespective of rates of decarboxylation, and that the radioactivity in solution around diseased tissue is relatively low at first but high during sporulation when rates of decarboxylation are low. This might suggest that heavily diseased tissue fails to take up IAA. Further studies of the uptake and metabolism of IAA by both healthy and diseased tissue, using more precise methods of separation of tissue components, will be reported in a subsequent paper. In all instances, the distribution of radioactivity during sporulation was similar to that of table II.

**Effect of Lighting on Decarboxylation and Rust Development.** In view of the significance given to the time of appearance of the initial burst of activity (29), it was desirable to establish the reality of the differences noticed between experiments 1 and 2 (fig 2). First considerations suggested that levels of infection (which are not easily reproduced at values greater than 100 pustules per leaf) might govern response during initial development. It also was noted that a dim source of incandescent light (less than 25 ft-c) was present during the light periods of experiment 1. Accordingly this variable was tested, employing much greater intensities of incandescent light. Two groups of plants were grown at 21°C but in separate rooms, one of which had some fluorescent lights replaced by 4 evenly-spaced 200 w, clear, incandescent lights placed approximately 4 ft above the plants. In contrast to the 1400 ft-c produced by a complete bank of fluorescents, there were 1100 ft-c of fluorescent light plus 200 from the incandescent bulbs. Seven days after planting, 9 hours after the start of the light period, plants from both light regimes were transferred for dusting to a room equipped with incandescent lights. After an additional 4 hours of fluorescent light in the incubation chamber followed by 11 hours of darkness, they were transferred back to the original light conditions. Tissue was collected for IAA decarboxylation assay 1 hour later. The complete protocol is presented in table III for assays on days 1 and 3, respectively.

Table II

| Distribution of Radioactivity from Carboxyl-labeled IAA for Healthy (H) and Diseased (D) Tissue. Data Expressed as Percentage of Initial IAA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tissue | 2 | 3 | 4 | Sporulation |
| CO₂ | H | D | H | D | H | D | H | D | H | D |
| Tissue | 56 | 59 | 57 | 56 | 56 | 56 | 56 | 56 | 56 | 56 |
| Bathing fluid | 29 | 18 | 31 | 23 | 28 | 27 | 28 | 27 | 28 | 27 |
| Total | 92 | 90 | 99 | 95 | 98 | 100 | 98 | 100 | 98 | 100 |
| CO₂ | 17 | 8 | 26 | 9 | 24 | 8 | 24 | 8 | 24 | 8 |
| Tissue | 52 | 43 | 48 | 44 | 50 | 40 | 50 | 40 | 50 | 40 |
| Bathing fluid | 34 | 51 | 30 | 44 | 38 | 57 | 38 | 57 | 38 | 57 |
| Total | 101 | 102 | 104 | 99 | 102 | 105 | 102 | 105 | 102 | 105 |

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which plants also received low intensity incandescent light.

Four additional experiments to check on this latter phenomenon are shown in table IV. The procedures and light conditions are similar to those described for the experiment of table III. During inoculation, the procedures for both groups were identical. In all instances the presence of strong incandescent light reduced the final number of infections, but with no discernible change in infection type (i.e. susceptibility or resistance). Visual examination suggested that infections under fluorescent lights developed more quickly. For example, in experiment 3, a count on the sixth day showed more than double the number of infections in the growth room with fluorescent lights, but not nearly as marked a difference 2 days later.

\textit{Effect of light during decarboxylation reaction.} Previous work (15,19,26,38) has suggested that plants grown under different photoperiods and in red or far-red light have different levels of IAA oxidase when the latter is determined in homogenates. Such studies have usually been undertaken several hours or days after the inducing conditions were imposed. The marked effects of exposing plants grown under incandescent lights to fluorescent lights for only 4 hours while in the chamber opened the question as to the effects of light on the decarboxylation process per se.

Preliminary experiments showed that light from incandescent sources with intensities of around 200 ft-c were inhibitory to \( \text{CO}_2 \) production from IAA. In an effort to determine if this effect arose from photosynthetic reutilization of \( \text{CO}_2 \) vessels were wrapped with DuPont cellophane as described for experiments in ascertaining the effects of red or far-red light on plant growth. The results of a number of experiments suggest that, although photosynthetic trapping of \( \text{CO}_2 \) released from IAA may account for a minor part of the inhibition, there are direct effects of light on IAA decarboxylation.

### Table III

\textit{Effect of Light Regime on IAA Decarboxylation. See Text for Details}

Tissue (0.5 g) and 50 \( \mu \)g of IAA were incubated (cpm 38,700) in 5 ml of 0.1 m \( \text{KH}_2\text{PO}_4 \) for the specified period. \( \text{CO}_2 \) was collected and assayed as described in Materials and Methods.

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Fluorescent</th>
<th>Incandescent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H*</td>
<td>C</td>
</tr>
<tr>
<td>cpmp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2230</td>
<td>2290</td>
</tr>
<tr>
<td>4</td>
<td>1910</td>
<td>1650</td>
</tr>
<tr>
<td>6</td>
<td>650</td>
<td>820</td>
</tr>
<tr>
<td>Total cpm</td>
<td>4790</td>
<td>4770</td>
</tr>
<tr>
<td>% decarboxylation</td>
<td>8.16</td>
<td>8.12</td>
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</table>

<table>
<thead>
<tr>
<th>Day 3</th>
<th>Fluorescent</th>
<th>Incandescent</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>C</td>
</tr>
<tr>
<td>cpmp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2310</td>
<td>2630</td>
</tr>
<tr>
<td>4</td>
<td>1430</td>
<td>1620</td>
</tr>
<tr>
<td>6</td>
<td>780</td>
<td>690</td>
</tr>
<tr>
<td>Total cpm</td>
<td>4520</td>
<td>4940</td>
</tr>
<tr>
<td>% decarboxylation</td>
<td>7.70</td>
<td>8.41</td>
</tr>
</tbody>
</table>

* Healthy bench control (H), healthy chamber control (C) and diseased (D) plants.

** Average of 3 replicates.

larly marked in the last third of the reaction period of 6 hours.

However, with plants grown under incandescent lights, there was a considerable difference between the bench and chamber controls on day 1. This difference had disappeared 2 days later. The change in lighting in the incubation chamber had an effect, which persisted for at least 18 hours, on the systems controlling decarboxylation of IAA. Rust development boosted the rate of decarboxylation by about 50% over the chamber controls, but of course by 300% over the bench controls in day 1. Light pre-treatment had an appreciable effect on the capacity of healthy leaves to metabolize IAA, but there was no extra effect on diseased tissue provided that chamber controls are used to express the relative increase due to infection. This experiment cannot be directly compared with experiments 1 and 2 in which there were no differences between controls but markedly different consequences of infection.

On the seventh day after inoculation, all treatments averaged 15% decarboxylation with no significant differences.

Despite the fact that in the present experiment all the plants were inoculated together, the fluorescent grown leaves supported over 200 pustules per leaf but the incandescent grown leaves averaged 155 pustules. This happened to be the same order of difference in pustule density as between experiment 2 with fluorescent grown plants and experiment 1 in

### Table IV

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Fluorescent</th>
<th>Incandescent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pustules per leaf</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>231</td>
<td>167</td>
</tr>
<tr>
<td>2</td>
<td>310</td>
<td>226</td>
</tr>
<tr>
<td>3 6 days*</td>
<td>190</td>
<td>80</td>
</tr>
<tr>
<td>8 days</td>
<td>220</td>
<td>150</td>
</tr>
<tr>
<td>4</td>
<td>123</td>
<td>99</td>
</tr>
</tbody>
</table>

* In experiment 1 and 2 pustules were counted about 10 days after inoculation. In experiment 3 at day 6 incipient sporulation occurred, on day 8 sporulation was nearly completed.
The Effect of Light during Assay of IAA Decarboxylation by First Leaf of Wheat

Tissue (0.3 g) was incubated in 5.0 ml of KH₂PO₄ containing 50 μg of IAA (67,000 cpm). Tissue was collected 30 minutes after the start of the light period, cut under the same light conditions, and placed in duplicate vessels wrapped with 2 layers of DuPont red or blue cellophane. The vessels were shaken in a transparent water bath with approximately 9 inches of water between the bottom of the vessels and bath. The temperature was regulated.

Table V contains typical results from a study of healthy wheat leaves collected from plants grown at 22° under a mixture of cool-white fluorescent and incandescent sources as described previously. All wrappings transmitted light beyond 700 μm (the far-red region), hence theoretical explanations in terms of a photoperiodic pigment are not warranted at the present time. Although data were obtained at hourly intervals, only the total for 4 hours is given in table V.

Table V

<table>
<thead>
<tr>
<th>Days after planting</th>
<th>% decarboxylation after 4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
</tr>
<tr>
<td>6</td>
<td>4.7</td>
</tr>
<tr>
<td>9</td>
<td>4.8</td>
</tr>
<tr>
<td>10</td>
<td>5.2</td>
</tr>
<tr>
<td>18</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Discussion

Although our results on decarboxylation of IAA at different stages of rust development are in general agreement with those of Shaw and Hawkins (29), they differ in some important details. The validity of their hypotheses concerning relationships between infection type and speed of change from high to low rates of decarboxylation as the rust develops is uncertain. However, it might be unwise to make strict comparisons between our respective results because of some differences in techniques.

One difference concerns the length of time for the measurement of decarboxylation. The selection of a 6 hour period in our work was based on a study of the kinetics of decarboxylation by both young and more mature leaves. It should be noted that this time period, relative to the 2 hour period of Shaw and Hawkins, should maximize differences between healthy and rusted tissue.

Another difference was that, although our plants were grown under controlled conditions of heat and light, we became aware that changes in illumination had effects on the reactions of healthy tissue. These variables are difficult to control in greenhouse studies and we do note that successive experiments were performed at different times of the year in Shaw and Hawkins' work. As important, perhaps, are the conditions of assay. The ratio of IAA to tissue may be a factor, since Goldsmith and Thimann (12) have evidence indicating that the rates of degradation of IAA by coleoptiles were dependent on the amount of applied auxin.

A major assumption in comparative daily examinations of healthy and diseased tissue is that uptake of substrate is equivalent throughout the experiment. The correctness of this assumption is questionable because of the differences in residual radioactivity observed in the bathing solutions. Although uptake of IAA is dependent on metabolism (1, 2), Bennet-Clark and Wheeler (2) and Blackman et al. (3) found accumulation of IAA to be a process possessing unusual kinetic features. The increased rates of metabolism previously demonstrated in diseased tissue do not mean necessarily that uptake by infected tissue will be greater or even the same as for normal, less metabolically active, tissues. The failure of diseased sporulating tissue to show the high rates of decarboxylation found in healthy tissue may depend on factors such as uptake or me-
tabolism of IAA by other routes. These aspects will be considered in another paper.

Despite limitations in the interpretation of decarboxylation data, it is clear that mechanisms other than inhibition of IAA oxidase must be considered in order to correlate studies of hormones in rust disease of cereals with other studies on disease-induced increases in auxin. When hormonal concentrations were determined quantitatively, it was possible to detect increases in IAA during mycelial growth (4, 6, 23, 24). The curves of figure 1 indicate that the levels of IAA would be much reduced by the oxidase during vegetative development of the rust fungus in wheat. Increase in auxin would require transport from uninfected organs or rapid synthesis of IAA by the host or parasite. The data of Srivastava and Shaw (33) suggest that synthesis of IAA by isolated cells of the parasite is too slow to be a factor.

It is possible, however, that the initial burst of decarboxylation may be concerned with parasite establishment through the development of fields of dominance as suggested by Shaw and Hawkins (29). In our study, the type of lighting influenced the density of pustules from standard inocula. A change in lighting also had a pronounced and somewhat persistent effect on the capacity of leaves to decarboxylate IAA. There are other reports that light is important during the invasion of the host plant by rust fungi (8, 21, 28). Maximum numbers of pustules were obtained only when a minimum of 500 ft-c of sunlight were given shortly after inoculation. It is possible that such effects may result from direct action of light on the IAA oxidase of the fungus (20), but Sharp et al. (28) did note that preinoculation light treatments were important.

Light activation of IAA oxidase in vitro is well known (9, 11, 37) and may play a role in vivo. More recently, there have been reports (10, 15, 19, 26, 38) of light effects on intact plants which can cause changes in the contents of cofactors or inhibitors of the enzyme. In these instances, red or farred irradiation or different photoperiods resulted in changes affecting enzyme activity in homogenates. The effects were measured at time intervals considerably after the imposition of effective light regimes, but they may explain differences in amounts of IAA in plants grown under different photoperiods or additional red light (22, 36).

It is difficult to determine from such data if the change in oxidase activity is a cause or result of change in growth habit. In addition, the principal documented effect (15, 19, 26) is a change in inhibitors acting as free radical traps. In the present work, the influence of light conditions can be observed almost immediately in vivo in the absence of any discernible effects on growth, although Downs et al. (7) have shown that irradiation from incandescent sources for extended periods can induce maturation of wheat more quickly than light from fluorescent sources. The immediacy of the response to light would appear to minimize a role for synthesis or destruction of cofactors of the type described by Mumford et al. (19) or Furuya et al. (10). Rather the levels of other regulatory compounds, such as phenols (13, 20, 25, 26, 32, 37) which might be readily altered by abrupt, 1 or 2 step changes in oxidation or reduction, appear to be a more useful system to explore.

It would seem premature to speculate on the role of IAA decarboxylation in the expression of resistance or susceptibility by the host. The susceptible host-parasite combination used in the present study showed variation equivalent to the difference reported for resistant and susceptible combinations (29). It is possible that under controlled conditions resistant combinations will still develop patterns of decarboxylation during infection which are distinct from those of susceptible reactions. We have not done such experiments because of a lack of suitable experimental material. It is not appropriate to compare different species of wheat hosts without first determining possible differential effects of environment on decarboxylation. The use of isogenic lines which differ by a single gene conditioning resistance would be more suitable and only now are they available in sufficient amounts for experimental purposes.

The present data post a set of questions concerning the regulation of IAA oxidase in normal leaves. It is possible that invasion by rust fungi disturbs the normal regulation of the enzyme so as to permit fungus establishment. It will be necessary to first establish the role of environmental factors, especially light, in regulatory processes before the relationships among IAA degradation, IAA accumulation and infection can be established.

Summary

A daily comparison was made of indoleacetic acid (IAA) degradation in the dark by healthy and rust-affected first leaf of wheat. Normal leaves showed a progressive increase in capacity to decarboxylate exogenous IAA when supplied at a concentration usually active in growth assays. With infected leaves, 3 phases could be distinguished. During the first 2 or 3 days after inoculation, there was a significant increase in IAA decarboxylation. Shortly before development of symptoms and continuing into the sporulation stage of fungus growth, the initially high rates of decarboxylation declined to the same level as the healthy controls. Only after sporulation was well underway did the rates of diseased tissue fall appreciably below the control rates. It is concluded that decarboxylation reactions, presumed to occur through an IAA oxidase, cannot account for increases in IAA during the early critical events of mycelial development of the stem-rust fungus in susceptible wheat. Although IAA accumulation during sporulation may arise from inhibition of the oxidase, analysis of tissue and exogenous concentrations of residual radioactivity suggest other alternatives for the data on decarboxylation rates during sporulation.
such as failure of IAA to be taken up by diseased tissues.

Since the initial burst of decarboxylation resulting from inoculation may be important for parasite invasion by reducing, rather than increasing, IAA levels in the host, it was examined in more detail. Light conditions during pre- and post-inoculation periods had a pronounced effect on the capacity of normal and infected tissue to decarboxylate IAA and thus alter the characteristics of the initial burst. The final infection intensity, but not resistance or susceptibility as usually defined, were altered by light regime.

Studies of blue, red, and far-red illumination indicate that decarboxylation rates may be regulated by co-factors or inhibitors controlled by a system similar to that described for photoperiod phenomena. Blue and far-red treatments, when effective, stimulated decarboxylation. Red irradiation usually inhibited, but occasionally in older tissue, stimulated rates after 2 or 3 hours exposure. Although the conditions influencing IAA decarboxylation have not been explored adequately, the data emphasize a need for caution in evaluating the role of IAA oxidase in the development of fungal infections.

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Literature Cited


