Induction of Disease Symptoms in Barley by Powdery Mildew

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Powdery mildews usually grow superficially on their hosts but produce symptoms of disease throughout the underlying host tissues. The symptoms include marked increase in respiratory rate (1, 3, 15, 24, 26, 30, 35); accumulation of organic and inorganic substances (1, 29, 36); increase in dry weight (10, 26); increase in wet weight (3); decrease in photosynthesis when the disease is well developed (1, 26, 29); chlorosis at mildew colony centers with redevelopment of chlorophyll at those centers in older colonies (1), and retention of green pigment around colonies on detached or senescent leaves (10, 29, 33). Similar symptoms occur in rust infected leaves (25, 29, 30, 31, 35, 36). The processes leading to the production of these symptoms are unknown. It has been suggested that diffusible substances produce the respiratory increase (1) and the accumulation phenomena (29), but there has been little evidence that such substances are involved.

The respiratory rates of mildewed and rusted tissues are quite high and several workers have prepared extracts of such diseased tissue in the hopes of finding substances which produce large respiratory increases in healthy tissue. Gretschushnikoff (11) reported respiratory stimulation by crude extracts of several varieties of rusted plants. He attributed the activity to urea and ammonia. More recently, Millerd and Scott (14) reported that phosphate buffer extracts of mildewed barley would produce respiratory stimulation of healthy tissue. Others have used similar methods and have found no consistent differences between extracts of healthy and diseased tissues in ability to stimulate respiration of healthy tissue (8, 9).

While respiratory increase is a symptom of many other plant diseases, only two compounds have been identified as the causative agents of such increase—ethylene (5, 37) and victorin (12, 13). Both compounds probably act as uncoupling agents and, as Allen concluded in review (2), the alterations in metabolism of plant tissue under attack by obligate parasites do not conform to the changes produced by uncoupling agents.

Auxins and other growth promoting compounds may be responsible for the respiratory changes or other symptoms of mildewed or rusted tissues. Such compounds accumulate in large amounts in infected tissues (7, 22, 23, 28), possibly as a result of a reduced ability to decarboxylate indole acetic acid (23, 28). It is very likely that the growth responses which occur in some hosts in response to rust infection are mediated in part by these accumulated growth substances. It has been suggested that the high levels of growth substances are also intimately involved in the development of symptoms other than growth (8, 28) but it is not yet clear what role is played by these substances.

In addition to the symptoms listed above, changes of a more degenerative nature may occur when host and parasite are incompatible. Possibly both the abrupt death of cells occurring in incompatible associations and the less degenerative changes of compatible associations arise from the same kinds of processes, as suggested by Mueller for the symptoms produced by some facultative parasites (17, 18). However, for neither compatible nor incompatible associations involving obligate parasites is there any significant information concerning the nature or origin of symptom-inducing substances; even the evidence for their existence is meager. We have tried, therefore, to obtain further evidence concerning the possible participation and role of diffusible agents in the induction of symptoms by an obligate parasite.

Powdery mildew was used because the superficial growth habit of this parasite offered important advantages for the proposed experiments. Living mildewed tissues as well as extracts of mildew or mildewed tissues were utilized as potential sources of symptom-inducing substances. These were tested in several ways for ability to produce symptoms of disease in healthy leaf tissue.

Materials & Methods

► Environment: Healthy and mildewed barley plants were grown in a controlled environment room. Light for plant growth was supplied by standard fluorescent fixtures (4 ft long) mounted horizontally in pairs with opposed reflectors 70 cm apart. Each fixture contained two 40 w cool white fluorescent tubes. The light intensity between the light fixtures where the seedlings were grown ranged from 610 to 650 ft-c as measured by a probe held vertically facing one of the light fixtures. One 150 w incandescent

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2 This paper is based on part of the Ph.D. thesis of the senior author. The work was made possible by the National Science Foundation through a graduate fellowship granted to the senior author.
3 Present address: Agricultural Botany Building, University of Minnesota, St. Paul 1.
bulb was suspended over each of the four pairs of light fixtures in the room. The light period was 15 hours, commencing daily at 2:00 AM. The temperature of the room averaged 21.5 °C when the lights were on and 20.5 °C during the dark periods. The relative humidity averaged 65 and 80 % in the light and dark periods, respectively. The cooling system in the room was activated approximately every half hour. During each cooling and heating cycle the temperature varied about one degree Centigrade, the relative humidity about 16 % units.

Host & Parasite: The barley varieties, Atlas⁴, Atlas 46, Oderbrucker, and the six differential varieties⁵ of Newton and Cherewick (19), were used as host material. The culture of Erysiphe graminis DC used during most of this investigation was a member of race three (USDA culture CR3⁶) which produced a type 4 reaction on Atlas and a type 0 reaction on Atlas 46 (Conventions of Newton & Cherewick (19]). Three other races of mildew obtained from natural infections in the greenhouse were used in initial experiments. The reactions of these races to their hosts will be given when pertinent.

A barley crop was seeded every 7 to 10 days in soil composed of half silt and half compost in 24 four-inch pots. Six to ten seeds were placed in a single row across each pot to facilitate inoculation of the seedlings. Even though healthy and mildewed plants were grown in the same controlled environment room, unwanted contamination with mildew was not serious. Uninoculated seedlings ordinarily had one to three mildew colonies.

Spore Harvest & Inoculation: The first leaves of seedlings were usually inoculated 10 days after seeding. The spores for inoculum were gathered from plants inoculated about 15 days earlier. Secondary colonization was abundant on such plants so that spores from both primary and secondary colonies were included in the harvest. The spores were shaken from the parent plants onto a piece of aluminum foil. These spores were then distributed onto new seedlings in a settling tower patterned after those of Bell et al. (4), Sharp et al. (27), and Petersen (21). Two pots of seedlings were inoculated with each spore shower in the tower.

Infection rates in the range 0.1 to 3.2 colonies/mm² were obtained by this method using spore charges of 2 to 100 mg. From counts of the numbers of spores per unit area which fell on slides placed next to the leaves and from the numbers of infections per unit leaf area, we estimated that 1.4 to 8.8 % of the applied spores produced infection. The higher infection rates were obtained from spores applied at low population densities. Regardless of density, the rates of infection from tower-dispersed spores were at least ten times greater than those from spores dispersed in water suspensions.

The plants were placed in the light immediately after inoculation. No special precautions were taken to maintain high humidity for newly inoculated plants. Inoculation took place during the last hour of the light period and, as described above, the relative humidity during the ensuing 9 dark hours was about 80 %.

Measurements of Mildew Colonies, Starch Areas, & Green Islands: Measurements of mildew colonies and associated areas of symptom development were made with an ocular micrometer in a dissecting microscope. Colony dimensions were taken from infected leaves which had been boiled 3 to 5 minutes in lactophenol-cotton blue, a treatment which makes the surface hyphae clearly visible (34). Areas of starch deposition were measured in leaves treated with I-KI solution after the chlorophyll had been removed by boiling the leaves in water and methyl alcohol successively. Green islands were measured after leaves were detached and incubated in moist petri dishes in the light of the controlled environment room. The islands became discernible 2 days after detachment and were clearly defined on the 3rd day. All sets of plants used for measurement of colonies or symptoms were inoculated between 4:00 and 5:00 PM. Measurements were then taken within 1 hour of 11:30 AM on the following and succeeding days.

Plant Extracts & Assays for Stimulation of Respiration: Extracts of mildewed and healthy plants were prepared using the technique of Miller and Scott (14). Heavily mildewed leaves (50–200 colonies/leaf) were harvested 4 to 7 days after inoculation. The leaves were frozen and then ground in 0.05 M KH₂PO₄ in a Waring blender. The resulting suspensions were heated 20 minutes in boiling water and then filtered through Whatman No. 1 filter paper. Final volumes of the extracts (in ml) varied from four to ten times the original wet weight of the leaves (in g).

Leaf extracts were applied to healthy leaf tissue in several ways. In one experiment the extracts were used as the suspending medium for healthy leaf discs in Warburg flasks. In other experiments the extracts were vacuum infiltrated into leaf discs before the discs were placed in the respirometer vessels. In yet other experiments, cut ends of leaves were placed in extracts for 17 hours before the discs were cut and the respiration measured. In all cases, simultaneous measurements were made of the respiration rates of tissues treated with 0.05 M KH₂PO₄.

Standard constant volume Warburg techniques were used to measure the oxygen uptake of the treated tissues. Each flask contained 3 ml of buffer or extract and fifty 5-mm leaf discs with filter paper and 0.2 ml of 20 % KOH in the center well. Oxygen uptake usually was measured over a 4 hour period. Measurements were made in the dark at a temperature of 25.0 ± 0.5 °C.

Junctions Between Seedling Leaves: Barley seeds were surface sterilized by exposure to a satura-

⁴ Supplied by J. M. Simmons, Department of Agronomy, University of California, Davis.
⁵ Supplied by Dr. J. G. Moseman, Crops Research Division, ARS, USDA, Beltsville, Md.
ate atmosphere of propylene oxide for 2 to 3 hours followed by a 20 minute dip in 1% sodium hypochlorite. The bleach treatment alone was sufficient to sterilize some batches of seed. The treated seeds were plated on nutrient agar and incubated for 4 to 5 days. The few non-sterile seeds were discarded and the remainder transferred in pairs to sterile test tubes (25 x 200 mm) containing 5 ml Hoagland’s solution (full or half strength). At 4 to 5 days the seedlings were still young enough to be pulled easily from the agar plates but were tall enough to extend out of the nutrient solution without special supports. At 7 to 10 days of age each seedling pair was taken from its tube and the leaves of the two seedlings clamped together back to back (fig 1). The clamps were made from No. 22 plastic insulated solid wire of such a size that sections of the leaf about two centimeters long were brought into contact. A layer of water agar about one millimeter thick was placed between the leaves in the area of contact. One exposed surface of the leaf pair was inoculated opposite the region of contact with spores applied by means of a blunt glass rod. These spores, harvested from the controlled environment room, were not necessarily free from extraneous micro-organisms. However, there was no gross contamination of the seedling pairs.

Junctions Between Detached Leaves & Leaf Tissues: Small tissue squares were cut from in and around mildew colonies with two No. 11 surgeon’s blades held rigidly side by side about 1.1 mm apart. Such tissue squares had been used in a study of the respiration rates of tissues under the influence of mildew colonies (6), in which it was found that the small tissues were relatively uninjured by the cutting and handling operations when appropriate care was taken. In the experiments reported here, such excised tissues were placed on the horizontal surfaces of detached leaves in petri dishes. In some instances the epidermis was removed from one side of the excised tissue and one side of the detached leaf so that the mesophyll tissue of both could be brought into contact (fig 3). In other tests, the epidermis was removed only from the detached leaf: the excised tissue square was placed on edge with its vascular system in contact with the exposed mesophyll of the detached leaf (fig 2). The detached leaves with superimposed tissues were incubated in the light in the controlled environment room.

Spore Extracts: Extracts were prepared from spores similar to those used for inoculum. Each spore crop (30-150 mg fr wt) was suspended in 3 to 10 ml water or 0.1 % Triton WR-1339*. After 2 to 3 hours the washings from the spores were filtered through No. 1 filter paper. The filtrates were boiled a few minutes and filtered again. Some spore

*Polymeric alkylaryl polyether alcohol, Rohm and Haas, Philadelphia, a surfactant of low toxicity.
suspensions were boiled before filtration. If the extracts were not used immediately they were taken to dryness at room temperature, restored to a volume equivalent to 1 ml of extract per 100 mg of spores extracted and stored in a freezer until needed.

Test drops of the extracts were placed on detached leaves in moist petri dishes which were then incubated in the light. Each drop, containing about five microliters of extract, was delivered from a small calibrated dropper onto a point between the mid-rib and edge of the leaf and about midway along the length of the leaf. In initial assays the epidermis was first removed in the test area but later the drop was placed over a puncture made with the tip of a surgeon’s blade. The covers were left off the petri dishes for about two hours after applying the test drops. This speeded entry of the drop as the leaf became slightly water deficient.

**Results**

Attention was first centered on the respiratory increase in mildewed tissues and the possibility that this symptom is produced by a soluble substance. Extracts of mildewed and healthy tissues were made by the method of Miller and Scott (14) and tested for ability to increase the respiratory rate of healthy tissues. Seven host-parasite combinations were used involving three races of mildew. These produced the following reaction types: 4 on Atlas and Oderbrucker, 3 on Atlas, 2 on Nepal, and 1 on Black Hulless. Extracts were prepared from healthy and mildewed leaves for each combination. The extracts were applied to susceptible barley leaves (susceptible to the race of mildew from which extracts were made) by one or two of the techniques described in Methods; the respiratory rate of the treated tissue then was measured. In addition, four of the extracts were applied to the Nepal variety which was resistant (type 2 reaction) to the mildew races used in this case.

The results varied. Four of the extracts, including those assayed on the resistant variety, produced no significant respiratory alteration compared to buffer alone. The assays that did produce respiratory alterations are listed in table I. Wherever an extract of mildewed tissue produced a respiratory increase, there was a similar increase produced by the corresponding extract from healthy tissue. Since the respiratory stimulation was not a special characteristic of extracts from the diseased leaves, this response was not necessarily the same as the respiratory increase occurring in mildewed tissue.

The search for agents which produce respiratory alterations in mildewed tissues was discontinued in favor of studies using symptoms which are more easily detected.

### Table II

**Pairings of Seedlings Used to Test for Symptom Induction in Healthy Leaves by Mildewed Leaves**

<table>
<thead>
<tr>
<th>Host Variety</th>
<th>Reaction of Host to Mildew</th>
<th>Test Variety</th>
<th>Treatment</th>
<th>Buffer alone</th>
<th>Extract of Healthy Leaf</th>
<th>Extract of Mildewed Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlas—Atlas</td>
<td><em>(X)</em></td>
<td>AH</td>
<td>26 37</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black Hulless—Black Hulless</td>
<td><em>(X)</em></td>
<td>AH</td>
<td>28 37</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black Hulless—Black Hulless</td>
<td><em>(X)</em></td>
<td>AH</td>
<td>40 46</td>
<td>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlas—Black Hulless</td>
<td></td>
<td>AH</td>
<td>40 53</td>
<td>50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*“X” indicates inoculated member

### Table I

**Effect of Extracts of Mildewed & Healthy Barley Leaves on Respiration of Healthy Leaf Tissues**

<table>
<thead>
<tr>
<th>Extract</th>
<th><strong>μl O₂/hr/50-5mm Leaf discs</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Host Variety</strong></td>
<td><strong>Reaction of Host to Mildew</strong></td>
</tr>
<tr>
<td>Black Hulless</td>
<td>1</td>
</tr>
<tr>
<td>Atlas</td>
<td>4</td>
</tr>
<tr>
<td>Atlas</td>
<td>4</td>
</tr>
<tr>
<td>Atlas</td>
<td>4</td>
</tr>
</tbody>
</table>
for any indication of an induced effect of one leaf on its partner. In pairs involving a resistant host, it was hoped necrosis might appear in the uninoculated member opposite an inoculated leaf. In combinations involving susceptible members only, it was thought that chlorosis of the type which occurs at mildew colony centers might be induced.

No symptoms occurred in Atlas in response to a mildewed opposite member. The presence of agar on the back of a mildewed leaf did not enhance the spread of chlorosis from individual colonies in that leaf or into the adjacent leaf. The results with Black Hulless were less conclusive. Necrosis occasionally developed opposite an inoculated leaf in this variety, but similar necrosis developed in uninoculated leaf pairs. This necrosis did not occur consistently and probably resulted from injuries received during the pairing operations. It was not possible to tell whether the necrosis which occurred in both members of a pair resulted from an induced effect by one member on the other or whether both members had suffered a common injury. The latter was more likely since necrosis in one member was often not accompanied by necrosis in the other.

The absence of any clear evidence for induction of disease symptoms between these artificially joined leaf pairs might reflect a general failure of movement of diffusing substances across the junctions. Tests were, therefore, carried out to determine if tracer substances would move across the artificial bridges. Neither 0.01% safranin nor 0.1% eosin applied to the roots of one member could be detected in the agar bridge or in the leaf of the opposite member. Similarly, $P^{32}$ did not move in great amounts across the bridges. This tracer was applied to the roots of one member of 23 pairs of plants: each received 0.001 or 0.002 mc of $P^{32}$ as phosphate ion. Ten days after application, counts were taken directly from 5 mm leaf discs cut from within and adjacent to the areas of leaf contact. While rates of more than 1,000 cpm/disc were obtained from treated plants, the highest obtained from an opposite member was 17 cpm. Considering the long period of incubation for these seedling pairs with $P^{32}$, these results were taken to indicate that $P^{32}$ was quite restricted in its movement from one leaf to the other. The failure of the dyes and of $P^{32}$ to move freely across the bridge does not necessarily mean that all classes of compounds would fail to move, but it seemed evident that either the bridges did not provide a suitable path for movement of diffusible materials or materials did not move out of the leaf in appreciable amounts. For these reasons the use of seedling pairs was discontinued.

Growth of Mildew Colonies & Associated Symptoms: In the experiments described in the preceding section neither chlorosis nor necrosis proved to be ideal for the purposes of this investigation. Chlorosis occurred late in the development of the colony while necrosis was easily induced by factors other than mildew infection and required the use of incompatible combinations of inoculum permitting

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**Table III**

**Average Daily Mildew Colony Dimensions**

2 to 12 Days After Inoculation

<table>
<thead>
<tr>
<th>Colony age, days</th>
<th>Colony length, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Series A*</td>
</tr>
<tr>
<td>2</td>
<td>...</td>
</tr>
<tr>
<td>3</td>
<td>...</td>
</tr>
<tr>
<td>4</td>
<td>0.56</td>
</tr>
<tr>
<td>5</td>
<td>...</td>
</tr>
<tr>
<td>6</td>
<td>...</td>
</tr>
<tr>
<td>7</td>
<td>2.30</td>
</tr>
<tr>
<td>8</td>
<td>2.76</td>
</tr>
<tr>
<td>9</td>
<td>...</td>
</tr>
<tr>
<td>10</td>
<td>3.84</td>
</tr>
<tr>
<td>11</td>
<td>...</td>
</tr>
<tr>
<td>12</td>
<td>5.44</td>
</tr>
</tbody>
</table>

* Series A inoculated 7 days after seeding, each point an average of ten or more measurements.
** Series B inoculated at 10 days, each value an average of at least 20 measurements.

Among the other symptoms produced by mildew, starch accumulation and green island formation seemed promising. Starch is detectable by merely treating decolorized leaves with IKI solution, while green islands become apparent if mildewed leaves are detached and incubated 2 to 3 days (fig 5).

Measurements of the zones showing these symptoms as well as the lengths of the mildew colonies themselves were made to learn the developmental patterns of the symptoms relative to the colony. Colony lengths and extent of starch deposition were measured on freshly harvested leaves, whereas the dimensions of green islands were taken from leaves detached 3 days after inoculation and incubated in the light (table III & fig 4). Mildew colony growth averaged

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**Fig. 4.** The length of mildew colonies and associated green islands and areas of starch deposition as a function of colony age. Green islands average of 10 measurements; starch areas average of 20 measurements; colony lengths average of plots of the data in table III.
0.56 mm/day from the 2nd through the 12th days and was nearly constant. The starch areas associated with such colonies did not form until the 3rd day, when they averaged nearly 2 mm in length while colony length averaged only 0.5 mm. From the 3rd day until the 7th day the starch areas enlarged at a rate slightly faster than the rate of elongation of the mildew colonies. The starch faded on the 8th day and was absent thereafter.

Thus starch deposition occurred at an appreciable distance beyond the advancing mildew hyphae. On the 7th day after inoculation, for example, the starch areas were 2.2 mm longer than the mildew colonies. This means that on each end of the colony there was an induced effect extending 1.1 mm ahead of the hyphal front. Since the hyphae grew 0.28 mm/day from each end of the colony (half the rate of total colony elongation), the induced effects occurred at least 3 days ahead of the advancing mildew.

As indicated in figure 4, the green islands produced around young mildew colonies on detached leaves were about the same size as the zones of starch deposition in attached leaves. Mildew development itself was not followed closely on these detached leaves, but the large size of the islands indicates this symptom occurred in tissues not yet covered by the surface hyphae. For example, 5 days after inoculation and only 2 days after detachment, the green islands were 3 mm long or about twice the length of mildew colonies on attached leaves.

The size of the green island relative to that of the mildew colony was shown indirectly by an experiment in which the mildew was removed at the time of leaf detachment (3 days after inoculation). The islands which subsequently developed averaged 2.5 mm long and did not change size significantly 2 to 6 days after removal of the hyphae. The colonies which produced these islands averaged only 0.5 mm long when they were removed from the leaves.

Such measurements indicate that green islands and starch accumulation are early symptoms of mildew development, appearing when the colony is still small and affecting tissues at an appreciable distance ahead of the advancing hyphae. These characteristics suggested that either symptom might well be utilized in the search for agents of symptom induction. We thought that green islands would provide a more practical criterion than starch accumulation since the detection of starch required sacrifice of the leaves and since starch accumulation was more transitory than the green islands around.

► Juncions Between Excised Tissues & Detached Leaves: In the assays for green island production it was necessary to use detached leaves. It was hoped that good contact might be made between these detached leaves and small squares of mildewed tissue excised from mildewed leaves. Squares about 1.1 mm on each side were cut from the centers of 6-day colonies and from the centers and peripheral regions of 10-day colonies. They were placed on the mesophyll of detached leaves (figs. 2 & 3) which were then incubated several days. About five tissues of each type were applied in this way, but in no case was there any evidence of an induction of green islands. The mildew continued to develop on the excised pieces, however, and in some cases sporulated heavily. Sometimes the tissues remained green where these spores fell upon the exposed mesophyll of the detached leaf. This indication that spores had produced green islands suggested the possibility of obtaining inducing substances directly from the spores.

► Spore Extracts: Aqueous spore extracts and washings were tested for green island inducing ability by applying 5-μl drops of the solutions to the horizontal surfaces of detached leaves. All of six different spore extracts produced green islands when tested in this manner.

The aspects of the islands varied with the concentration of the extract. With solutions containing the extract of 10 to 20 mg spores/ml the extracts induced islands 5 to 15 mm long. The effects of these applications became evident after 5 to 7 days' incubation. With material from 80 to 100 mg spores/ml a wide chlorotic band was induced around the green islands and the islands became apparent after only 3 days (fig 6). The islands, often 20 mm in length, were produced irregularly. Sometimes there was no green island at the center of the extensive chlorotic area.

![FIG. 6 (lower). Green island and chlorosis produced by high concentrations of spore extracts. A. Residue of test drop. B. Green island induced by extract. C. Green islands from contaminating mildew colony. D. Chlorotic area induced by extract.](https://www.plantphysiol.org/)

![FIG. 5 (upper). Green islands on detached mildewed leaves 8 days after inoculation. Leaves detached 2 days after inoculation.](https://www.plantphysiol.org/)
Areas of starch accumulation also developed in detached leaves treated with spore extracts. These areas usually corresponded in position and extent with the region later covered by the green island. The starch formed within 1 day of treatment and faded in an irregular fashion 2 to 3 days thereafter. When high concentrations of spore extract were applied, starch occupied that region which later became chlorotic, as well as the central green area.

The spore extracts themselves turned black when placed on the mesophyll of leaves. The more concentrated extracts (100 mg spores extracted per ml) became intensely black, while such extracts diluted one to ten darkened only slightly. Extracts incubated under similar conditions on glass plates did not blacken.

The action of spore extracts on resistant hosts was similar to their action on susceptible hosts. A single preparation containing the extract of 77 mg spores per ml was tested concurrently on two resistant varieties, Atlas 46 and Chevron, as well as on the susceptible Atlas used routinely in other assays. Green islands and starch deposits developed similarly on all three hosts. The action of spore extracts does not, therefore, appear to be related to the degree of compatibility between host and parasite.

A short period of washing was sufficient to remove considerable activity from the spores. One crop of spores (150 mg fr wt) was washed 20 minutes in 7.5 ml of 0.1 % WR-1339, filtered, and resuspended in an equal amount of WR-1339. The spores were filtered 16 hours later, resuspended, and kept for another 24 hours before a final filtration. Each filtrate was taken to dryness. The 20 minute wash weighed 23 mg, the 16 hour wash 23 mg, and the final 24 hour wash 9 mg. Thus 15% of the fresh weight of the spores was lost in each of the first two washings. After being redissolved in water, all three extracts produced green islands. The materials from the last wash had the least activity while those from the 20 minute and the 16 hour washes had equally strong activity.

The spore extracts were treated in a variety of ways without appreciable loss in activity. These treatments included boiling before or after spore removal, freezing, and storage in the frozen condition for several weeks.

The green island inducing activity was not reduced by treatment with strong ion-exchange resins. Aliquots of an extract (100 mg spores extracted per ml) were mixed with one or the other of two ion-exchange resins, Dowex-1 in the chloride form or Dowex 50 in the sodium form (quaternary ammonium styrene anion exchange resin & sulfonic acid styrene cation exchange resin, respectively, Dow Chemical Co.) To 0.5 ml portions of the resins was added 1/5 ml of extract. After removing the resins by filtration, the filtrates were restored to the original volume of 0.2 ml. These solutions still produced green islands, indicating that the active materials in the extracts were not charged. The anion-exchange resin did, however, remove the light brown color of the extract.

Two preliminary chromatographic treatments of the spore extracts were carried out. Onto each of two strips of No. 1 Whatman chromatography filter paper (2.5 inches wide) were spotted 50 ml of spore extract (100 mg spores/ml). One strip was developed with water, the other with water-saturated formic acid-butanol (1-8) with ascending solvent. The strips were dried, cut into five parts, and each part eluted separately. Activity in the chromatogram developed with formic acid-butanol was in the eluate from the segment including the origin, whereas the activity from the paper developed in water was from the segment of Rf 0.5. In both chromatograms the green island inducing activity was associated with a UV fluorescing spot. The activity recovered from these chromatograms was about one-tenth that in the solution originally placed on the paper, as judged from the size of the green island produced.

Since the activity was located at one spot on each chromatogram, it is associated either with a single substance or a group of substances with closely related properties. The active materials are relatively polar since they did not move in the non-polar butanol-formic acid solvent. This fact was also indicated by a separate test which showed that no activity was lost from an aqueous spore extract when it was partitioned against diethyl ether.

The material responsible for the black reaction on leaf mesophyll was separated from the green island inducing material on the chromatogram developed with butanol-formic acid.  

Table IV

<table>
<thead>
<tr>
<th>Material</th>
<th>Concns. Applied</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole acetic acid</td>
<td>4.0, 0.4 &amp; 0.04 mg/l</td>
<td>-</td>
</tr>
<tr>
<td>Maleic hydrazide</td>
<td>100.0, 100.0, 10.0, 1.0, &amp; 0.1 mg/l</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.0, 0.5 &amp; 0.05 g/l</td>
<td>-</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>87.0*, 8.7, &amp; 0.9 g/l</td>
<td>-</td>
</tr>
<tr>
<td>Yeast extract**</td>
<td>50.0 &amp; 5.0 g/l</td>
<td>+</td>
</tr>
<tr>
<td>Rust uredospore extract</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

* Killed tissue in test area.  
** Difco Laboratories, Detroit, Mich.

Exchange of rust spores was also tested using the facilities of the Crops Research Division, ARS, USDA, at the University of Minnesota, St. Paul. The senior author found that nickelous ion, cobaltous ion, kinetin, or benzimidazolone will produce green islands and localized starch accumulation.

In further experiments using the facilities of the Crops Research Division, ARS, USDA, at the University of Minnesota, St. Paul, the senior author found that nickelous ion, cobaltous ion, kinetin, or benzimidazolone will produce green islands and localized starch accumulation.
duced islands about one centimeter long surrounded by wide regions of induced chlorosis much like the effects produced by high concentrations of mildew spore extracts. At 5 g/l the islands were 3 to 4 mm long, well defined, but with no induced chlorosis. At this concentration the action of yeast extract was much like that of mildew spore extracts at about 10 mg spores/ml. The extract of rust uredospores containing the extract of 50 mg spores/ml also had activity equivalent to that of mildew spore extracts at about 10 mg/ml.

Discussion

Respiratory Stimulation: Extracts of healthy leaves stimulated respiration of leaf tissue much as did extracts of mildewed tissues (Table I). Similar results were obtained by Miller and Scott (Miller, personal communication) in work subsequent to their report that extracts of mildewed tissue alone had such activity (14). In both laboratories results were variable; not all extracts have the ability to increase the respiration of healthy tissues. While the source of this variation is unknown, extracts of healthy and diseased tissues are essentially similar in activity. There is no evidence, then, that the respiratory increase following infection results from the action of materials of the type contained in these plant extracts. To establish with certainty that the activities of extracts and mildew are similar or dissimilar would require a careful qualitative comparison of the oxidative metabolism of mildewed tissue and tissue treated with plant extracts.

It is not yet known if spore extracts will produce respiratory stimulation of healthy leaf tissue. Since the respiratory responses resulting from mildew infection occur later in the development of infection than does green island formation or starch deposition (6), it is unlikely that the agents causing the latter symptoms will also produce respiratory stimulation.

Symptoms Other Than Respiratory Increase: Mildew hyphae may produce host responses through the action of materials of the type contained in spore extracts. This is indicated by the facts that at least two of the symptoms of the disease are produced by spore extracts (green islands & starch deposits), and that these symptoms occur some distance ahead of growing mildew hyphae. It is reasonable to expect that symptoms occurring at a distance from the parasite result from the action of diffusible substances. At the same time, an important characteristic of these inducing substances is the restricted spread of their activity around the point of application. Symptoms produced by both spore extracts and mildew hyphae are definitely localized.

Shaw and Hawkins (28) have aptly described the local effect of infection by obligate parasites as the establishment of a "field of dominance" which results in the accumulation of organic and inorganic materials in the infection court. It has not been clear how the field is established, although it has seemed likely that the large amounts of organic material in the tissues are involved in some way. Superficially, at least, it appears that such a field of dominance is produced by the mildew spore extracts. However, it is not yet known whether or not there is transport into tissues treated with spore extract since starch deposition alone does not necessarily depend on such movement. It is likely that such transport does occur; indeed it may be found that the primary activity of the extracts is a re-direction of the transport system of the leaves.

It is quite possible that the spore extracts contain growth substances and that these mediate the responses in the host tissues. Certain growth regulating substances have been reported as producing green islands and other localized effects in detached leaves. For example, Osborn and Hallaway (20) found green retention in those parts of detached senescent cherry leaves treated with either indole-3-aceto nitrile or the n-butyl ester of 2,4-D. The protein nitrogen in the treated zones was maintained at high levels relative to the untreated portions of the leaves. Kinetin was not effective in their tests. Kinetin did, however, have a localized effect on pea stem segments in tests by Thimmann and Laloraya (32), producing high levels of protein nitrogen in the treated parts. Mothes et al. (16) also found localized effects of kinetin; treated portions of tobacco leaves remained green after detachment and soluble nitrogen compounds accumulated in the treated zones. The localized effects of these growth substances support the speculation that mildew spore extracts contain naturally-occurring growth substances which elicit the responses leading to green island formation.

There is no direct evidence that the substances obtained from mildew spores are present and operative in mildewed tissues. The fact that excised mildewed tissues did not produce green islands on detached leaves suggests that inducing materials, if present, are not in active or mobile form. It may be that they are incorporated at specific sites in the host cells during the processes leading to symptom expression. Such a mechanism has been postulated by Thimmann and Laloraya (32) to explain the localized effect of kinetin.

It is not clear why spore extracts at high concentration produced chlorosis around the green islands. It is possible that more than one type of material was acting; one producing chlorosis, the other the green island. On the other hand, both effects may be produced by the same agent since starch accumulation sometimes occurred in the regions which became chlorotic as well as in those which became green islands.

Production of chlorosis concentrically around a green central area is characteristic of the type 2 reaction of wheat to stem rust (31). This reaction may be of the same type as that produced by high concentrations of spore extract. The possibilities in this area are yet to be explored.

From the limited information available, it appears that the green island inducing materials in mildew spore extracts are water soluble, un-ionized, non-volatile and stable at the temperature of boiling water.
Whether or not the chlorosis-producing materials are the same is yet to be determined. Whether or not the green island inducing materials in yeast extract or rust spore extracts have the same characteristics is likewise not known. The occurrence of similar activity in rust, yeast, and mildew extracts suggests that the active material may occur generally in fungi. It remains to be shown if the activity is due to materials known to control processes in higher plants.

If it is established that mildew produces symptoms in its host by means of materials such as those contained in mildew spore extracts, it would greatly simplify the study of host-parasite physiology. The effects of the parasite could then be simulated in part by chemical means and studied in the absence of the parasite. The development of the parasite in turn could be studied when presented with the chemically altered host. The symptom inducing materials in spore extracts warrant additional investigation.

**Summary**

Several methods were used to learn if diffusible substances produce any of the symptoms of mildew infection in barley leaves. The possibility that such substances are responsible for the respiratory increase in infected tissue was examined by testing the effect of phosphate buffer extracts of healthy and mildewed leaves on the respiration rate of healthy tissue. Some stimulation of respiration occurred, but activities of extracts from healthy and mildewed leaves were similar. There was no reason to assume, therefore, that the extracts altered the metabolism of the leaf in the same way as do mildew colonies.

The investigation turned to the use of visible symptoms and a search for evidence that diffusible substances are involved in their production. Pairings of leaves were made in such a way that mildewed tissues were brought into contact with healthy leaves. There were no clear indications that necrosis or chlorosis was induced in the healthy leaves by mildewed tissue regardless of what combination of susceptible and resistant varieties was employed.

From a study of symptom development around individual mildew colonies it was found that starch accumulation in attached leaves occurs at distances of the order of 1 mm beyond the hyphal tips of growing mildew colonies and that equally large green islands develop around colonies on detached leaves. Green islands were not induced in healthy leaves when small excised squares of mildewed tissue were placed on the mesophyll of detached leaves. On the other hand, water extracts of mildew spores did induce green islands and starch accumulation. At high concentrations these extracts induced wide chlorotic bands around the central green islands. The extracts could be boiled, dried, frozen, partitioned against diethyl ether, or treated with ion-exchange resins without loss in green island inducing activity. Green islands were also produced by yeast extract and by an extract of uredospores of wheat stem rust.

We concluded that mildew hyphae may alter their hosts through the action of substances of the type contained in the spore extracts. More evidence is needed to establish this with certainty.

**Literature Cited**


