HOST-PATHOGEN RELATIONS IN POWDERY MILDEW OF BARLEY

III. UTILIZATION OF RESPIRATORY ENERGY

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Summary

A quantitative comparison was made of the respiratory response, and concurrent changes in the concentrations of nucleic acid and protein, following infection of barley with the powdery mildew fungus (*Erysiphe graminis* var. *hordei*). Infection of a susceptible barley variety resulted in increased respiration and increased amounts of ribonucleic acid phosphorus (RNAP) in the host tissue. Infection of etiolated tissue of a susceptible variety was not accompanied by increased respiration or by increased RNAP levels. The increased respiration in resistant barley varieties following infection was not associated with increased RNAP levels. It is suggested that the metabolic responses following infection are different in susceptible and resistant varieties.

I. INTRODUCTION

An increased rate of respiration accompanying infection of plant cells by a wide range of pathogens is well documented (reviewed by P. J. Allen 1953, 1954, 1959; Uritani and Akazawa 1959; Eberhardt 1960; Millerd and Scott 1962). The significance of this increased respiration in the overall disease symptoms can be assessed only when the mechanism of this respiratory response is understood.

As an approach to this problem, a quantitative comparison was made of the respiratory response and concurrent synthesis following infection of barley with the powdery mildew fungus (*Erysiphe graminis*).

II. MATERIALS AND METHODS

The barley varieties investigated were Prior, susceptible; 463‡ (B69§), semi-resistant; and 659‡ (B278§), highly resistant to the pathogen *E. graminis* var. *hordei* Marchal. The fungus was cultured on the susceptible variety, Atlas (185‡). The plants were grown in the greenhouse (15–23°C) and inoculated as described by White and Baker (1954) 6–8 days after sowing of seed and when the primary leaf was 6·5–7·5 cm long. Only the top 5 cm of the primary leaf was used in all assays. For experiments with etiolated tissue, seedlings were grown in complete darkness and inoculated in semi-darkness. Preparation of this material for assays was carried out under conditions of low light intensity.

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‡ Waite Agricultural Research Institute accession number.

§ B numbers refer to number in University of Sydney varietal accession lists.

Measurements of oxygen consumption by the standard Warburg technique were carried out as described previously (Millerd and Scott 1956).

Using 2-g samples of leaf material, nucleic acids were estimated according to Smillie and Krotkov (1960). The final volume of the perchloric acid extract was 25–35 ml. Using 1-ml portions, deoxyribonucleic acid (DNA) was estimated according to Burton (1956) and, after acid digestion and conversion to orthophosphate, total nucleic acid phosphorus was estimated by the procedure of R. J. L. Allen (1940). Protein determinations on the residues after extraction of nucleic acids were calculated from the nitrogen content following Kjeldahl digestion.

Surface area was determined with an airflow planimeter (Jenkins 1959). For dry weight determinations, samples (1 g) were dried at 102°C.

All estimations were carried out in triplicate.

The progress and extent of infection was checked by histological examination following clearing and staining with lacto-phenol containing 1% cotton blue (White and Baker 1954).

III. Results

Using barley varieties showing different responses to powdery mildew fungus, an examination was made, following infection, of the respiratory rate, the levels of DNAP, RNAP, and protein, and the dry weight.

(*a*) *Susceptible Variety*

Prior barley is a variety susceptible to infection by powdery mildew fungus. Following infection, a marked respiratory rise was observed (Table 1). In addition, considerably higher levels of ribonucleic acid phosphorus (RNAP) were observed in infected tissues when the results were expressed on a fresh weight basis. Over the period 2–6 days after inoculation, the increases observed were significant at $P < 0{\cdot}001$.

Since the method of expressing results is frequently of great significance, both DNAP and dry weights of tissues were determined. Examination of leaves stained with Feulgen revealed that, at the time of inoculation, cell division had ceased in the portions of the leaf used for experiment. The DNAP content of these plants can thus be considered a valid index of cell number for polyploid cells rarely occur in young tissue.

As shown in Table 1, non-infected and infected leaves contain the same amount of DNAP and dry matter per gram fresh weight in the first 4 days after inoculation. Thus, during this time period, the increases in respiratory rate and RNAP level of infected tissue are also apparent when expressed on the basis of DNAP ($P < 0{\cdot}001$ 2 and 4 days after inoculation) or dry weight ($P < 0{\cdot}01$ 2 days and $P < 0{\cdot}001$ 4 days after inoculation).

It is difficult to make a true comparison of non-infected and infected tissues, since infected tissues consist of both leaf and fungal cells. It was not possible to achieve a complete separation of host and pathogen, but in advanced stages of infection (e.g. 6 days after inoculation) the hyphae could be removed by brushing with a camel-hair brush. In agreement with earlier observations on wheat infected

with powdery mildew (P. J. Allen and Goddard 1938), microscopic examination following such treatment showed that only haustoria remained. When such a separation was made, the higher RNAP level of infected tissue (significant at $P < 0{\cdot}001$ on the basis of fresh or dry weight, or DNAP) was still observed (Table 1).

In separate experiments, surface area determinations were made on leaves 6 days after inoculation. The surface area per unit fresh weight and hence per unit DNAP was the same in both non-infected and infected leaves; a decrease in surface

TABLE 1

EFFECT OF POWDERY MILDEW INFECTION ON RESPIRATORY RATE, DRY WEIGHT, RNAP, DNAP, AND PROTEIN CONTENT OF PRIOR BARLEY

Condition of Green Tissue*	Oxygen Uptake (μl/hr/g fresh wt.)	RNAP (μg/g fresh wt.)	DNAP (μg/g fresh wt.)	Dry Weight (mg/g fresh wt.)	Protein (mg/g fresh wt.)
Non-infected	412	259	26	89	24·7
Non-infected	314	202	19	90	26·2
Infected (2)	395	220	19	92	25·9
Non-infected	261	149	19	86	24·6
Infected (4)	434	179	19	88	22·4
Non-infected	210	127	20	87	22·8
Infected (6)	668	153	19	106	19·9
Non-infected	151	63	27	75	17·6
Infected (8)	403	125	30	97	17·7
Non-infected†	285	96	16	83	
Non-infected†‡	240				
Infected (6·5)†	650	145	17	92	
Infected (6·5)†‡	575	137	17		

* No. of days after inoculation given in parenthesis.

† From a separate experiment.

‡ Tissue brushed with camel-hair brush.

area per unit dry weight was observed (Table 2). However, there was a significant ($P < 0{\cdot}001$) increase in RNAP in infected tissue whatever basis of expression was used.

As shown in Table 2, RNAP content of these infected leaves was 36% higher on a fresh weight basis. The increase observed was significant at $P < 0{\cdot}001$ when the results are expressed on the basis of fresh or dry weight or DNAP. In no instances were the increases observed in the RNAP content of infected tissues accompanied by marked changes in protein content (Tables 1 and 2).

Since the energy relationships of photosynthetic tissues are complex, a study was made of the effect of powdery mildew infection on etiolated leaves of a susceptible

barley strain. As is seen in Table 3, the results are quite different from those observed following infection of green tissue (Tables 1 and 2). There was no increase in the RNAP content of infected tissue, and in most experiments there was only a slight

TABLE 2

EFFECT OF POWDERY MILDEW INFECTION ON RESPIRATORY RATE, DRY WEIGHT, RNAP, DNAP, AND PROTEIN CONTENT OF PRIOR BARLEY 6 DAYS AFTER INOCULATION

Results are for surface areas of 52 cm^2 per gram fresh weight of non-infected and infected leaves

Condition of Green Tissue	Oxygen Uptake (μl/hr/g fresh wt.)	RNAP (μg/g fresh wt.)	DNAP (μg/g fresh wt.)	Dry Weight (mg/g fresh wt.)	Protein (mg/g fresh wt.)
Non-infected	211	97	26	81	17·9
Infected	541	132	28	94	17·5

increase in respiratory rate. Etiolated tissue was less uniform than green tissue and more variation was obtained between replicate assays. The variation observed in the examination of the respiratory response was probably also due to the difficulty

TABLE 3

EFFECT OF POWDERY MILDEW INFECTION ON RESPIRATORY RATE, DRY WEIGHT, RNAP, DNAP, AND PROTEIN CONTENT OF ETIOLATED PRIOR BARLEY

Condition of Etiolated Tissue*	Oxygen Uptake (μl/hr/g fresh wt.)	RNAP (μg/g fresh wt.)	DNAP (μg/g fresh wt.)	Dry Weight (mg/g fresh wt.)	Protein (mg/g fresh wt.)
Non-infected	371	278	35		
Infected (2)	381	276	34		
Non-infected	302	208	33		
Infected (4)	354	188	33		
Non-infected†	315	188	33	82	15·7
Infected (4)†	332	188	34	90	16·0

* No. of days after inoculation given in parenthesis.

† From a separate experiment.

of maintaining complete etiolation, even slight greening of the material resulted in the characteristic respiratory response following infection. Histological examination showed that the pattern of infection of etiolated tissue was similar to that observed with green tissue.

By calculation from results shown in Tables 1 and 3, the rate of respiration in etiolated tissue is, on a per cell (DNAP) basis, only two-thirds that of green tissue. The possibility exists that respiratory rate in this type of tissue is limited by substrate availability. The factors limiting the respiration of green and of etiolated barley leaves were compared. With both non-infected and infected etiolated tissue, oxygen consumption was increased 15–20% by the addition of 5×10^{-5}M 2,4-dinitrophenol (DNP). This is approximately the level of increase usually observed with green tissue and DNP (5×10^{-5}M). The presence of glucose (10^{-2}M) in the incubating medium had no effect on the oxygen consumption of green tissue and caused an increase (*c.* 15%) with etiolated tissue. With both green and etiolated tissue, glucose (0·1M) resulted in a considerable (*c.* 30%) increase in oxygen consumption.

(*b*) *Semi-resistant Variety*

The effects of infection on a semi-resistant variety were examined (Table 4). Because resistance is associated with death of mesophyll cells in the host (White and Baker 1954), these results should be considered in association with the histological state of the tissues.

TABLE 4

EFFECT OF POWDERY MILDEW INFECTION ON RESPIRATORY RATE, DRY WEIGHT, RNAP, DNAP, AND PROTEIN CONTENT OF A SEMI-RESISTANT BARLEY VARIETY

Condition of Green Tissue*	Oxygen Uptake (μl/hr/g fresh wt.)	RNAP (μg/g fresh wt.)	DNAP (μg/g fresh wt.)	Dry Weight (mg/g fresh wt.)
Non-infected	387	187	29	95
Infected (2)	569	177	28	96
Non-infected	245	113	30	89
Infected (4)	756	185	40	156

* No. of days after inoculation given in parenthesis.

The results obtained with the infected susceptible barley variety may be contrasted with those of the semi-resistant variety in the early stages of disease development. Two days after inoculation, while the respiratory rise is observed in both varieties, there is a significant decrease ($P < 0{\cdot}05$ on the basis of fresh or dry weight) in RNAP content in the infected semi-resistant variety (Table 4). The same trend is apparent if the results are expressed on the basis of DNAP as an index of cell number in the two tissue types. Four days after inoculation, infected leaves were distorted and the tips markedly necrotic. As shown in Table 4, at this time an increase in dry weight per unit fresh weight was observed in infected leaves, most probably resulting from extensive cell dehydration. If the results are expressed on the basis of dry weight, no increase in RNAP is observed in the infected tissue (Table 4). If the results are expressed on the basis of DNAP, an increase ($P < 0{\cdot}001$) in RNAP is observed.

(c) *Highly Resistant Variety*

Inoculation of a highly resistant barley strain by powdery mildew fungus is followed, within 2 days, by death of host cells and complete cessation of fungal growth within 4·3 days (White and Baker 1954). As shown in Table 5, there is, on the basis of fresh weight or DNAP, a significant decrease in RNAP content at 1 ($P < 0{\cdot}001$) and 4 ($P < 0{\cdot}05$) days after inoculation and a significant increase ($P < 0{\cdot}05$) after 2 days. On a dry weight basis, no significant changes in RNAP levels are observed 2 and 4 days after inoculation. On a fresh or dry weight basis, a significant ($P < 0{\cdot}001$) increase in RNAP was observed 6 days after inoculation (Table 5). However, if the results are expressed on a DNAP basis, or on a protein

TABLE 5

EFFECT OF POWDERY MILDEW INFECTION ON RESPIRATORY RATE, DRY WEIGHT, RNAP, DNAP, AND PROTEIN CONTENT OF A HIGHLY RESISTANT BARLEY VARIETY

Condition of Green Tissue*	Oxygen Uptake (μl/hr/g fresh wt.)	RNAP (μg/g fresh wt.)	DNAP (μg/g fresh wt.)	Dry Weight (mg/g fresh wt.)	Protein (mg/g fresh wt.)
Non-infected	410	250	28		20·9
Infected (1)	558	232	28		21·6
Non-infected	440	203	26	88	22·4
Infected (2)	524	213	26	89	25·9
Non-infected	398	193	25	89	22·6
Infected (4)	480	181	25	91	21·8
Non-infected	257	112	23	91	16·3
Infected (6)	325	130	27	91	20·4

* No. of days after inoculation given in parenthesis.

basis, no increase in RNAP is observed. As in the case of the semi-resistant barley strain (Table 4), the infected tissue contained in the later stages of infection more cells per unit fresh weight, as shown by the higher (significant at $P < 0{\cdot}001$) DNAP and protein content, than did a comparable sample of non-infected tissue.

IV. Discussion

In susceptible hosts infected by obligate parasites, there have been a number of instances of increased synthetic activity associated with increased respiratory rate (e.g. Yarwood and Cohen 1951; Samborski and Shaw 1956; Daly and Sayre 1957; Mukherjee and Shaw 1962). The activities investigated include accumulation of substrates at sites of infection, growth of infected tissue, and increases in hormonal concentration.

In infections of barley leaves by powdery mildew fungus, the respiratory response was examined by correlating respiratory rate with the synthetic activity of the host cells as shown by the levels of DNAP, RNAP, protein, and dry weight.

The most striking effect observed was in the RNA content of the host cells of a barley variety susceptible to infection by the powdery mildew fungus. As is seen in Table 1, when the results are expressed on the basis of DNAP (as an index of cell number), fresh weight, or dry weight, the RNAP content of non-infected tissue decreased with time. Such a decrease of RNA concentration with age has been reported previously (e.g. Gates and Bonner 1959; Smillie and Krotkov 1961). Within 2 days after inoculation, the RNA content of infected leaves was, on a fresh weight, dry weight, or DNAP basis, approximately 10% higher than that of non-infected tissue, and approximately 20% higher 4 days after infection. These increases were not accompanied by increased levels of protein.

It is not possible to separate completely the host and fungus, but following removal of the hyphae, the RNAP content of the infected tissue was little affected (Table 1). These results show that increased RNAP content of infected tissue was not due to the presence of fungal hyphae.

Marked changes in RNA content and turnover have been noted in rust infections of susceptible varieties of wheat (Rohringer and Heitefuss 1961; Mukherjee and Shaw 1962). The increased level of RNAP in barley leaves infected with powdery mildew is of particular significance in relation to the observation (Scott and Smillie 1962) that the activities of many enzymes in the host tissue are markedly increased following such infection.

The effect of powdery mildew on etiolated susceptible barley was examined. A comparison of the factors limiting the respiratory rate of green and of etiolated barley showed that, as would be expected, the respiration of etiolated tissue does demonstrate a greater limitation by substrate concentration than does that of green tissue, but the respiratory patterns of the two tissue types are not markedly different. However, the effects of powdery mildew infection of etiolated tissue are quite different from those observed with green tissue (Table 3). With etiolated tissue, there was no increase in RNAP content and little or no increase in respiration [the latter observation has been confirmed with other barley strains (Scott and Smillie 1963)]. It is apparent, then, that infection by powdery mildew fungus and an increased respiration of the host are not obligatorily coupled. Further investigations (Scott and Smillie 1963) have indicated that respiratory increases in infected susceptible barley varieties are related to changes in photosynthetic activity.

The effects of powdery mildew infection on barley varieties semi-resistant and highly resistant to this fungus were examined (Tables 4 and 5). Expression of results per unit DNAP, indicative of comparable number of cells in non-infected and infected tissues, showed the following: a marked increase in oxygen consumption after infection in both varieties, but no increase in RNAP in the semi-resistant variety 2 days after inoculation, and no marked increase in RNAP in the highly resistant variety within 6 days following inoculation.

These investigations have shown that infection of a susceptible barley variety by powdery mildew fungus resulted in increased respiration and increased levels of

RNAP in the host tissue. The increased respiration observed in resistant varieties, following infection, was not accompanied by increased RNAP levels. This observation suggests that the mechanism of this respiratory response may be different from that occurring in infections of susceptible varieties.

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