HOST PATHOGEN RELATIONS IN POWDERY MILDEW OF BARLEY

II. CHANGES IN RESPIRATORY PATTERN

By ADELE MILLERD* and K. SCOTT†

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Summary

The histological nature of resistance of barley seedlings to infection by *Erysiphe graminis* var. *hordei* is well understood. Infection by this obligate parasite causes marked biochemical changes in the host. These changes are expressed in an increased respiratory rate. The pattern of respiratory change is different and typical for the varietal types tested and may be correlated with the observed histological changes. This increased respiration may well be due to the formation, either by the pathogen or by the host pathogen combination, of an agent which uncouples directly or indirectly the concomitant phosphorylations normally limiting the respiratory rate.

I. INTRODUCTION

Wheat seedlings infected with *Erysiphe graminis* var. *triticii* have been shown (Allen and Goddard 1938) to respire at a rate much higher than that of non-infected wheat of the same age. This increase in respiration was shown to be localized in the mesophyll tissue although it is not necessarily in immediate contact with the invading ectoparasite. Several possible explanations of this changed respiratory pattern can be proffered (Allen 1953), including the presence of an uncoupling agent, produced either directly by the pathogen or by the host-pathogen combination. It is established that the factor frequently limiting respiration is the rate of concomitant phosphorylations (Potter and Recknagel 1951; Lardy and Wellman 1952). There is one physiological respiratory rise occurring in higher plants which has been explained on the basis of the formation of an uncoupling agent. This is the climacteric rise in respiration which accompanies the ripening of some fruits (Millerd, Bonner, and Biale 1953).

Histological studies on host-pathogen relations in powdery mildew of barley (White and Baker 1954) indicate that resistance in barley to infection by *E. graminis* var. *hordei* Marchal is a function of the rapidity and degree of mesophyll collapse rather than being the result of death of epidermal cells or aborted development of haustoria. White and Baker (1954) found that within 30 hr after inoculation an haustorium was formed in each infection court of all types of barley. In highly resistant barley varieties, mycelial growth was restricted, haustoria were fewer, and mesophyll collapse more rapid (30-48 hr after inoculation) than in resistant, semi-resistant, and susceptible varieties. In these three variety groupings during the first 54 hr after inoculation, the amount of mycelial growth and the number of haustoria formed per infection court were the same. There was no collapse of mesophyll cells during this period. In the resistant hosts, mesophyll collapse commenced at 54-72 hr after inoculation. After cell collapse had commenced haustoria and mycelial development were

* Department of Biochemistry, University of Sydney.

† Faculty of Agriculture, University of Sydney.

restricted. In semi-resistant hosts, there was a rapid collapse of mesophyll cells 72-90 hr after inoculation. Coincident with this collapse, as in resistant varieties, there was a restriction of haustoria and mycelial development. In the susceptible varieties there was unrestricted development of haustoria and mycelia during the first 90 hr and no mesophyll collapse.

The purpose of this investigation was to try to establish whether the premise of the formation of an uncoupling agent could be correlated with these histological findings.

II. Materials and Methods

The barley varieties investigated were B49 "Kinver", susceptible; "Cape", susceptible; B167, resistant; B69, semi-resistant; and B278, highly resistant to the pathogen, *E. graminis* var. *hordei* Marchal. The plants were grown and inoculated 7 days after planting according to the procedure of White and Baker (1954), the greenhouse temperature range was 25-30°C unless otherwise stated.

Table 1
OXYGEN CONSUMPTION OF NON-INFECTED TISSUE

Variety	Age (days after planting)	Experiment No.						
		1	2	3	4	1	2	3
		Oxygen Consumed per 60 Min (μl)				Oxygen Consumed per 120 Min (μl)		
Kinver	8	64	64	59		117	119	111
Cape	8	48	45	46	51			

Respiration studies were conducted at 30°C using the standard Warburg technique. The vessels were covered with black cloth to eliminate photosynthesis. The leaves were cut in 1-cm lengths. In each experimental vessel 200-mg aliquots were floated in 5×10^{-2}M potassium dihydrogenphosphate. The vessels were allowed to equilibrate for 15 min, the taps closed, and readings taken at 15-min intervals. All figures for gas exchanges represent the mean of two or three samples.

III. Results

It was first necessary to ascertain whether infection of barley by powdery mildew affected the leaf respiration in a manner similar to that observed with infected wheat (Allen and Goddard 1938). A number of experiments were conducted to characterize barley leaf respiration and to establish optimal conditions for respiration measurements.

(a) *Examination of Respiration of Barley Leaves*

(i) *Magnitude of Respiration.*—Two hundred mg of non-infected leaf tissue, which showed an oxygen consumption of *c.* 60 μl/hr, was used in all experiments.

TABLE 2

EFFECT OF ADDED SUBSTRATE ON THE RESPIRATORY RATE OF BARLEY LEAF, VARIETY B69, AGE 9 DAYS*

Substrate	Concentration (M)	Oxygen Consumed per 60 Min (μl)	Oxygen Consumed per 120 Min (μl)
—	—	143	268
Sucrose	10^{-2}	139	274
Glucose	10^{-2}	144	256
Citrate	10^{-2}	138	275
Succinate	10^{-2}	139	245

* The tissue used in this experiment was grown at a greenhouse temperature of 18°C. The higher respiration rate observed is due to the fact that the tissue is relatively young. Similar results have been obtained with tissue grown at 25-30°C.

(ii) *Variation in Oxygen Consumption of a Number of Samples Taken from One Batch of Seedlings.*—Replicate measurements of oxygen consumption of a number of samples showed good agreement as is seen in Table 1.

TABLE 3

EFFECT OF 2,4-DINITROPHENOL ON THE RESPIRATION OF BARLEY LEAF, VARIETY B69, AGE 9 DAYS

2,4-Dinitrophenol Concentration (M)	Oxygen Consumed per 60 Min (μl)	Increase (%)
—	90	—
10^{-3}	85	−6
3×10^{-4}	139	54
10^{-4}	114	27
3×10^{-5}	110	22
10^{-5}	91	1
3×10^{-6}	84	−7

(iii) *Effect of pH.*—Respiration studies were made at pH 4·5, 5·5, 6·0, and 7·0. No significant differences in oxygen consumption were observed. Vacuum infiltration of the buffers did not alter this finding.

(iv) *Effect of Added Substrates on the Rate of Respiration.*—Since the rate of respiration of tissues may be limited by the availability of substrate, it is interesting to note that the rate of respiration was unaffected by added substrates (Table 2). Vacuum infiltration of the substrates did not alter this result. The fact that added substrates caused only very small increases in the respiration of leaf tissue indicated that, in the non-infected leaf, substrate was non-limiting.

(v) *The Effect of Dinitrophenol on Respiration Rate.*—That the leaf respiration was controlled however by coupled phosphorylation may be shown by the effect of 2,4-dinitrophenol (DNP). DNP at 3×10^{-4}M concentration caused a 54 per cent. increase in respiratory rate (Table 3).

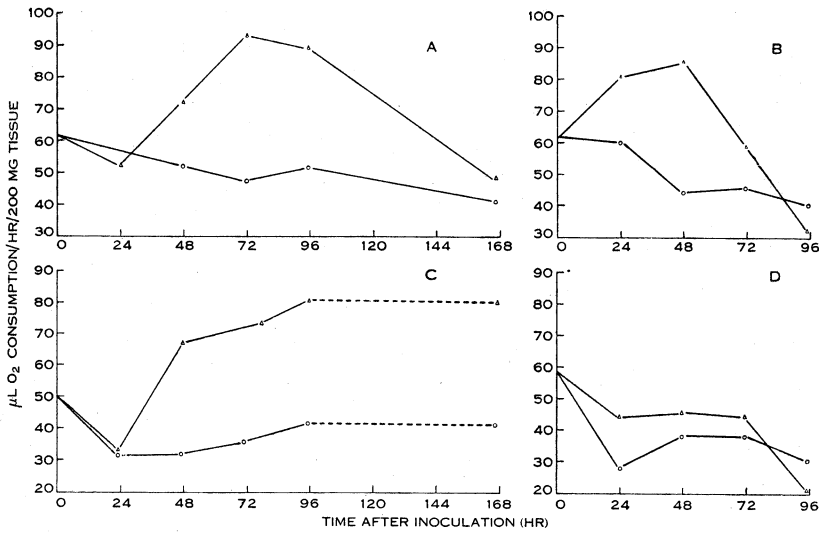

Fig. 1.—Comparison of respiration of non-infected and infected barley leaf. *A*, strain B69, semi-resistant. *B*, strain 278, highly resistant. *C*, strain B49, susceptible. *D*, strain B167, resistant. ○ Non-infected tissue. △ Infected tissue. - - - - From another experiment (see Fig. 1*C*).

(*b*) *Increase in Respiration of Barley Leaf after Inoculation with* E. graminis *var.* hordei

(i) *Respiration of Non-infected and Infected Barley Leaves.*—Normally the rate of respiration of barley leaf tissue, expressed on a wet weight basis, showed a gradual decrease with increasing age of the tissue. However, as is shown in Figure 1*A*, the rate of barley leaf respiration increased markedly after inoculation with *E. graminis* var. *hordei*. The increased oxygen consumption is accompanied by a corresponding increased carbon dioxide evolution (Table 4) indicating an increase in true respiration after infection. The marked increase in respiration was observed within 48 hr although in the semi-resistant strain B69 no histological evidence of the pathogen can be detected until 72 hr after inoculation.

The increased respiration observed would actually be much larger if the results were expressed on a surface area basis, since 200 mg fresh weight of non-infected

B69 tissue corresponded to 11·37 cm^2 (mean of three determinations) and 200 mg fresh weight of infected tissue corresponded to 10·05 cm^2 (mean of three determinations).

TABLE 4

COMPARISON OF OXYGEN CONSUMPTION AND CARBON DIOXIDE EVOLUTION OF NON-INFECTED AND INFECTED BARLEY LEAF TISSUE VARIETY B69, AGE 12 DAYS, 96 HR AFTER INOCULATION

Tissue Type	Oxygen Consumed per 60 Min (μl)	Carbon Dioxide Evolved per 60 Min (μl)
Non-infected	66	78
Infected	126	139

The tissue used for the experiments recorded in Tables 4 and 5 was grown in May 1955 with a greenhouse temperature of approximately 18°C. The lower growing temperature makes it necessary to inoculate at a later stage (8 days in these cases). Growth of both seedling and fungus is slower, but the relationship of respiration and histological changes remains the same: it is merely spread out over a longer period of time.

(ii) *Localization of Increased Respiration.*—The increased respiration observed on infection is not due to the respiration of the attached fungus. The fungus may be removed without apparent injury of the host by brushing with a camel hair brush (Allen and Goddard 1938). After B69 infected tissue is so treated only a few haustoria

TABLE 5

COMPARISON OF RESPIRATORY RATES OF NORMAL AND MILDEWED BARLEY LEAF TISSUES VARIETY B69, AGE 12 DAYS

Tissue Type	Oxygen Consumed per 60 Min per 200 mg Tissue (μl)		
	Expt. 1	Expt. 2	Expt. 3
Non-infected leaf	68	65	
Mildewed leaf	124	128	
Same leaf, mildew removed	110	112	116

remain, yet the respiration of the treated infected tissue is almost as high as that of untreated infected tissue (Table 5). It was not possible to extend these studies on the localization of increased respiration as was done by Allen and Goddard as it proved extremely difficult to strip off undamaged mesophyll-free epidermis from barley leaf.

(*c*) *Studies on Changes in Leaf Respiration of Four Varieties of Barley after Inoculation with* E. graminis *var.* hordei

The differential response of barley varieties to infection may be clearly demonstrated histologically. It is of interest, therefore, to determine if varietal differences are also expressed in the respiratory patterns following infection.

To study this problem various barley varieties were planted, and after 7 days one-half the seedlings were inoculated. The respiration of the non-infected and infected tissues was measured at 24-hr intervals. The results are shown in Figures 1*A-D*. All the barley varieties investigated have been tested at least twice, and no experiments conducted showed any variation in the curve characteristic for a particular variety although the absolute increases differed.

As is shown graphically the presence of the pathogen resulted in increased respiration and in all cases this increase precedes any detectable histological change. The changed respiratory pattern is, however, different and typical for each barley variety tested. The respiration increase occurred earliest (Fig. 1*B*) in the highly resistant strain B278 where the mesophyll tissue is most sensitive and collapses most rapidly. Infection of the semi-resistant strain (B69) resulted (Fig. 1*A*) in increased rate of respiration 48 hr after inoculation, which continued high until collapse of the cells and cessation of parasite growth. In the susceptible strain (B49), the onset of increased respiration (Fig. 1*C*) was delayed until 48 hr and the increase continued gradually as more cells became invaded by the parasite. The resistant strain B167 shows a cytological picture rather different from a state intermediate between semi-resistant and susceptible. It is of interest therefore that this was also true of the respiratory picture on infection (Fig. 1*D*). The histological picture shows odd cells collapsing from 30 hr after inoculation with the main cell collapse being observed at 72 hr. A respiratory rise occurred 24 hr after inoculation and a small increased respiration was maintained as long as fungal development continued. This relatively flat curve of increased respiration may be interpreted as being the sum of two processes—the increased respiration of progressively affected cells together with a trend towards normal respiration as these cells gradually collapse.

IV. Discussion

In highly resistant barley strains which will not tolerate the growth of the parasite *E. graminis* var. *hordei*, there was a very rapid biochemical response to the presence of the ectoparasite. A particularly rapid rise in respiration occurred followed by early collapse of the affected cells and a return of the respiratory rate to normal levels. In the susceptible strain, the onset of the response to the pathogen was less dramatic and the increased respiration continued parallel with fungal growth. Resistant and semi-resistant barley strains showed on infection respiratory patterns in accord with their recorded cytological behaviour. In these two the respiratory pattern was correlated with the time and extent of cell collapse. In all instances, increased respiration occurred in advance of cell collapse. Complete cell collapse and cessation of fungal growth, as occurs in highly resistant strains, resulted in a return to normal of the respiratory rate. In resistant strains where cell collapse takes place over a long period of time as mesophyll tissue becomes progressively affected by the fungus, the

small increased respiration was maintained. This respiratory pattern can be explained as being the resultant of two opposing processes. As cells collapse there was a tendency of the respiratory rate to return to normal and this was counteracted by the increased respiration resulting from continuing fungal growth which affects further leaf cells.

The nature of this increased respiration can be established. The increase cannot be due to the respiration of the attached fungus, since infected tissue with the fungus removed respired at almost the same rate as tissue with the fungus attached. That the rate of respiration of non-infected barley leaf tissue was not limited by the level of substrate was demonstrated. Thus the increased respiration cannot be explained by the suggestion that, on infection, more substrate was somehow made available to the cells. However, from the effects of DNP on barley leaf respiration it is apparent that respiratory rate was limited by the capacity of the phosphorylative system. When the respiration was artificially uncoupled from its concomitant phosphorylations, the rate was increased approximately 60 per cent.

The removal *in vivo* of the limitation of the phosphorylative system can be achieved in two very different ways. Firstly, either the rate of turnover of the phosphate acceptor, adenosine diphosphate (ADP), may be increased by an increased rate of synthetic reactions utilizing adenosine triphosphate (ATP), or the amount of ADP could be increased; both would lead to the formation of increased amounts of ATP and thus enable increased rates of synthesis. Secondly, either the rate of turnover of ADP could be increased by the liberation or formation of ATPase which would break down ATP immediately it was formed, or an agent might be formed which would uncouple the phosphorylative processes prior to the formation of ATP. The two uncoupling procedures are degenerative processes and would lead to disorganization and finally collapse of the cell.

The respiration of non-infected barley leaf is limited by the capacity of its phosphorylative system. The increased respiration observed on infection precedes any detectable histological changes but is later accompanied in highly resistant, semi-resistant, and resistant strains by marked degenerative changes leading finally to collapse of affected cells. In an infected susceptible strain, the marked increased respiratory rate is maintained for a long period of time but is not accompanied by cell collapse. The presence of the pathogen does, however, cause marked changes in the growth pattern—growth is stunted and limited, crop yield (if the plants survive long enough) is reduced, and the leaves die prematurely. All these are degenerative changes and may be interpreted, at least in part, by the hypothesis that here too uncoupling occurs but in this instance it is partial rather than absolute. This uncoupling could occur either directly by the formation of an agent with properties like DNP, or indirectly by the formation of a toxic agent causing a disorganization within the cell leading to the liberation of ATPase.

It would appear then that all the recorded changes, both biochemical and histological, may be explained by the postulate that the presence of the pathogen or the host-pathogen combination results in the formation of an agent which, either directly or indirectly, uncouples barley leaf respiration from its concomitant phosphorylations. Investigations are planned to elucidate the nature of this agent.

V. Acknowledgments

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