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The effect of powdery mildew (*Blumeria graminis* f. sp. Hordei) on compensatory photosynthesis and dark respiration in the uninfected fourth leaf of infected cultivated and wild barley lines

ABDELLAH AKHKHA

Biology Department, Faculty of Science, Taibah University,
Madinah Munawwarah, (Kingdom of Saudi Arabia).

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ABSTRACT

The compensatory photosynthesis in uninfected fourth leaf of infected plants was investigated in cv. Prisma and the two wild lines B19909 and I-17-40. Infection of the three lower leaves increased the rate of photosynthesis as well as the quantum efficiency in the powdery mildew tolerant line B19909 only. This indicates that compensatory photosynthesis may play an important role in tolerance of the parasite.

Key words: *Blumeria graminis,* compensatory photosynthesi, wild barley lines.

INTRODUCTION

Livne (1964) reported that photosynthesis was stimulated in uninfected leaves of heavily infected rusted bean plants. Similarly, photosynthesis in the upper, uninfected leaves of powdery mildew infected barley and pea plants, was also reported to be stimulated significantly (Ayres, 1981; Williams & Ayres, 1981; Walters & Ayres, 1983). Recent work on wild and cultivated oat lines, showed that the rate of photosynthesis in adjacent uninfected parts of infected leaves was reduced by powdery mildew infection but not to the same extent as photosynthesis in the infected tissues (Sabri, 1993).

These increases, referred to as compensatory photosynthesis, are possible mechanisms whereby the plant can compensate for photosynthates lost to the parasites in infected tissues. Compensatory photosynthesis could thus be a component of tolerance.

In the present investigation, compensatory photosynthesis and dark respiration were measured in the uninfected fourth leaves on plants whose lower three leaves had been inoculated and in plants in which the lower leaves were not inoculated of wild barley lines I-17-40 and B19909 and cv. Prisma.

MATERIAL AND METHODS

Plant material & growth

The lines of wild barley (*Hordeum spontaneum)* and of cultivated barley (*Hordeum vulgare*) used in this study were obtained either from the John Innes Centre, Norwich Research Park or from the Scottish Crop Research Institute, Invergowrie, Dundee.

Seed germination was carried out following Akhkha *et al*. (2003a) procedure by treating wild lines seeds with 7 days of chilling temperature at 4°C. Seeds of both wild and cultivated lines were germinated in damp filter paper then sown in peat based potting compost (Levington Horticulture Ltd.). Before use, the wild lines were inbred as mentioned in Akhkha *et al*. (2003a).

Plant growth

Grain germination and plant growth

Cultivated barley

Grains were germinated on damp filter paper in trays and incubated in the growth cabinet for four days before transplanting into the required growth medium.

Wild barley

Initial treatments were required to break the dormancy of the grain. Grains were placed on filter paper moistened with distilled water in Petri dishes and allowed to imbibe for half an hour. The dishes were then wrapped in plastic film to conserve moisture, and placed in a refrigerator at 4°C for 7 days. After chilling, the vernalised grains were germinated in the same way as the cultivated barley grains. This method gave high percentage germination.

After germination, both cultivated and wild barley seedlings were transplanted into pots as required.

For measurements of photosynthesis, germinated seedlings of both wild and cultivated lines were planted in 12.7 or 15-cm plastic pots containing Levington potting compost. Two seedlings were planted per pot. A week later, the seedlings were thinned to one per pot all of equal size or left as two per pot as required and any non growing seedlings were replaced. No supplementary feeding was carried out as most of the experiments lasted five to six weeks only.

Grain germination and plant growth

All the experiments were carried out in the same growth cabinet at a temperature of 20°C ± 2°C. The cabinet was illuminated during a 16h photoperiod by Kolorarc high-pressure mercury vapour lamps giving 130 µmol quanta m^{-2} s^{-1} of PAR at plant level. The relative humidity within the cabinet ranged between 65% and 80%.

Experimental design

The pots in the growth cabinet were arranged in a randomised design with three or four replicates and re-randomised at weekly intervals to ensure even growth.

Measurement of photosynthetic oxygen evolution

Photosynthetic rates were determined polarographically using a Hansatech LD2 leaf disc O_2 electrode (Hansatech Ltd., Paxman Road, Hardwick Industrial Estate, King's Lynn, Nofolk, UK) designed by Delieu and Walker (1981, 1983). The methods used followed that of Akhkha *et al*. (2001, 2003b).

Chlorophyll analysis

Chlorophyll concentration was determined in extracts made using hot methanol as described by Hipkins and Baker (1986), since hot methanol was found to result in rapid chlorophyll extraction.

Healthy and infected leaf samples used for photosynthesis measurements were cut into small pieces before placing in 10-ml methanol in 15 ml centrifuge tubes to allow the solvent to penetrate the tissues. The tubes were wrapped in aluminium foil to avoid light-induced breakdown of chlorophyll, and placed in a water bath heated to 60°C for 40 minutes. The chlorophyll/methanol solution was allowed to cool to room temperature and then centrifuged at 1500 rpm for 5 minutes in order to remove the leaf tissues. The supernatant was poured into a 15-ml volumetric flask and made up to a known volume. Chlorophyll content was determined spectrophotometrically using methanol as a standard at 650 and 665 nm.

From the specific absorption coefficients given by Mackinney (1941) for chlorophyll a and b in methanol, equations similar to those used for 80% acetone solutions (Arnon, 1949) have been derived (Holden, 1965):

$$\text{Chlorophyll a } (\text{mg } l^{-1}) = 16.5 \times D_{665} - 8.3 \times D_{650}$$

$$\text{Chlorophyll b } (\text{mg } l^{-1}) = 33.8 \times D_{650} - 12.5 \times D_{665}$$

$$\text{Total Chlorophyll } (\text{mg } l^{-1}) = 25.5 \times D_{650} - 4.0 \times D_{665}$$

Statistical analysis

The means and standard errors (shown in graphs) were calculated using Excel (Microsoft Office 2000). Analysis of variance was performed using Minitab's ANOVA and General Linear Model (version 13).

RESULTS

Effects of infection on photosynthesis

Maximum gross and net photosynthesis

Maximum rates of gross (Pg_{max}) and net (Pn_{max}) photosynthesis per unit leaf area or per milligram of chlorophyll, in the uninfected fourth leaf of inoculated and uninoculated plants are plotted in Figs. 1A-C. There were no significant differences in Pg_{max} per unit leaf area (Fig. 1A) between infected and uninfected plants up to eight days after inoculation. By the eleventh day after inoculation, the rates of maximum gross photosynthesis were higher in the uninfected fourth leaves of infected than in the same leaf on uninfected plants of both cv. Prisma and line B19909, but the difference was

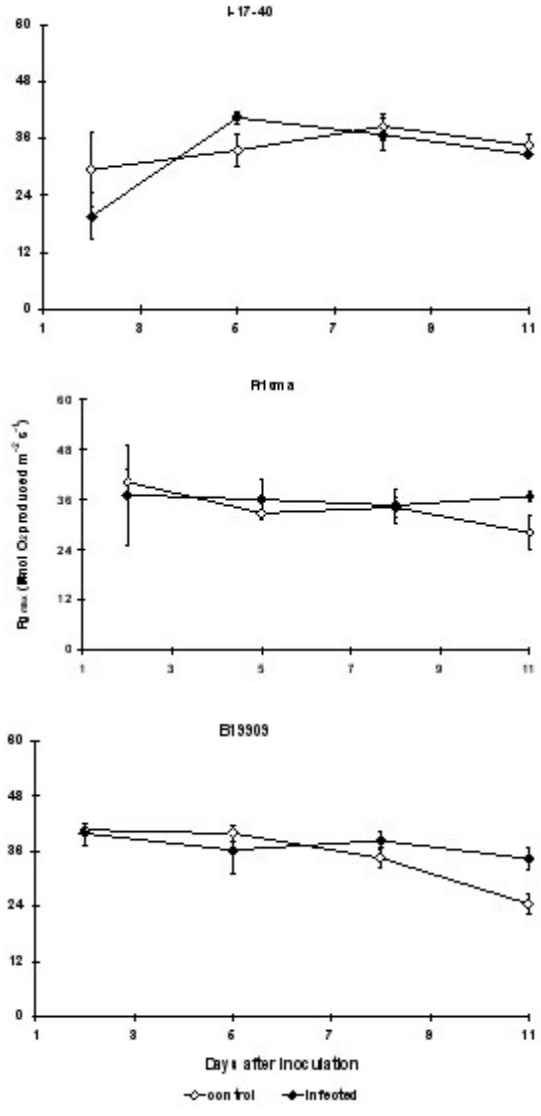

Fig. 1(A): The rates of gross photosynthesis per unit area in fourth leaves of three barely lines with three leaves infected by mildew. Each datum points is the mean of three replicates

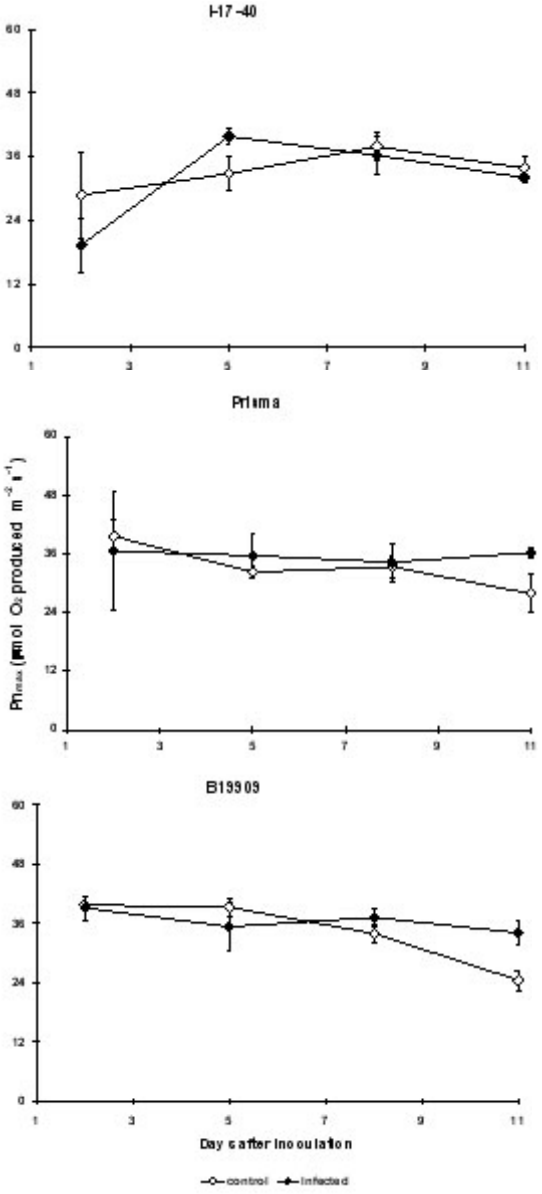

Fig. 1(B): The rates of net photosynthesis per unit area in fourth leaves of three barely lines with three leaves infected by mildew. Each datum points is the mean of three replicates

only significant ($p < 0.05$) in line B19909. In contrast, infection of the lower three leaves did not affect photosynthesis in the uninfected fourth leaf of line I-17-40 at any time during the experiment.

The effects on Pn_{max} per unit leaf area followed essentially the same pattern as on Pg_{max} in all three lines (Fig. 1B).

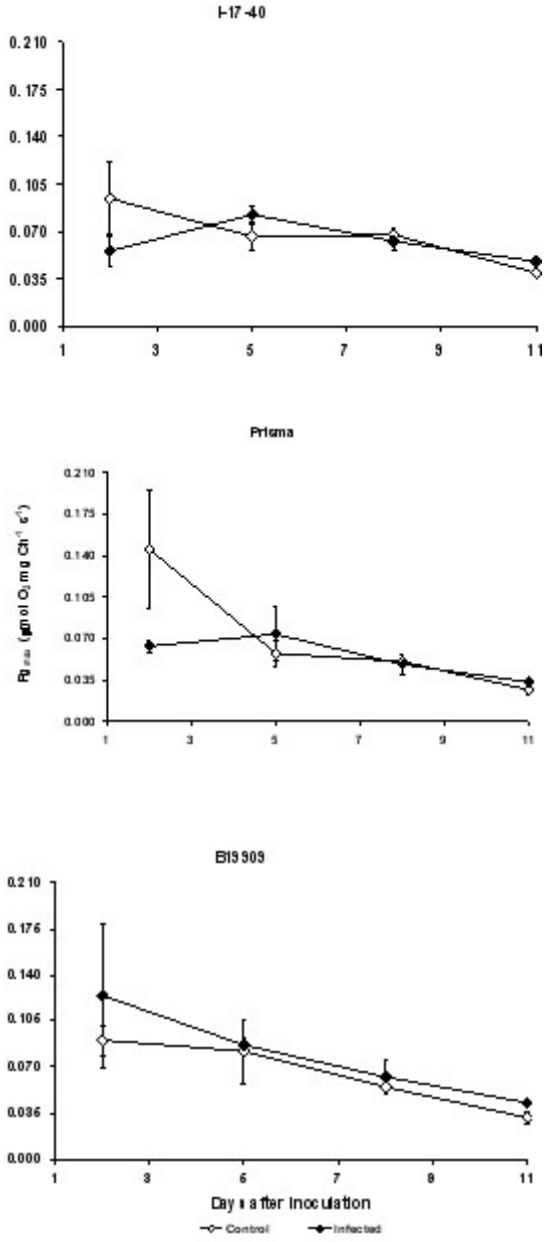

Fig. 1(C): The rates of gross photosynthesis per unit area in fourth leaves of three barely lines with three leaves infected by mildew. Each datum points is the mean of three replicates

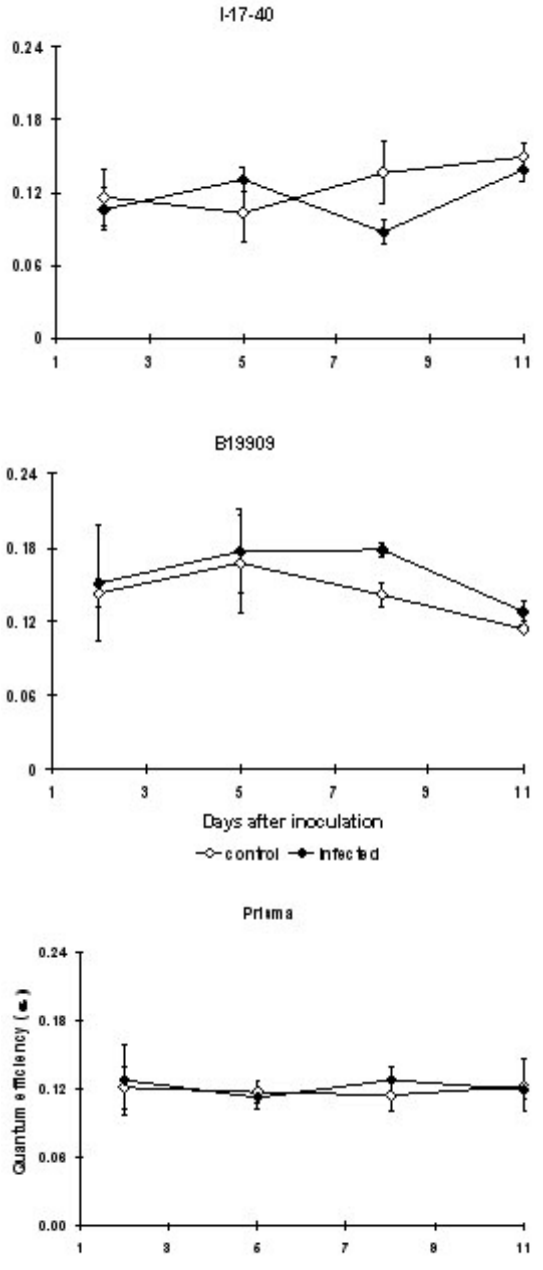

Fig. 2(A): The Quantum efficiency of photosynthesis in the fourth leaf of three barley lines whose lower three leaves were infected by mildew. Each datum point is the mean of three replicates

These results suggest that in line B19909, infection of the first three leaves stimulated photosynthesis in uninfected leaves to compensate for the photosynthetic losses which occurred in infected tissues.

When related to chlorophyll levels, the maximum rates of gross photosynthesis in the uninfected fourth leaf of infected plants were not significantly different from those in the fourth leaf of the uninfected plants (Fig. 1C).

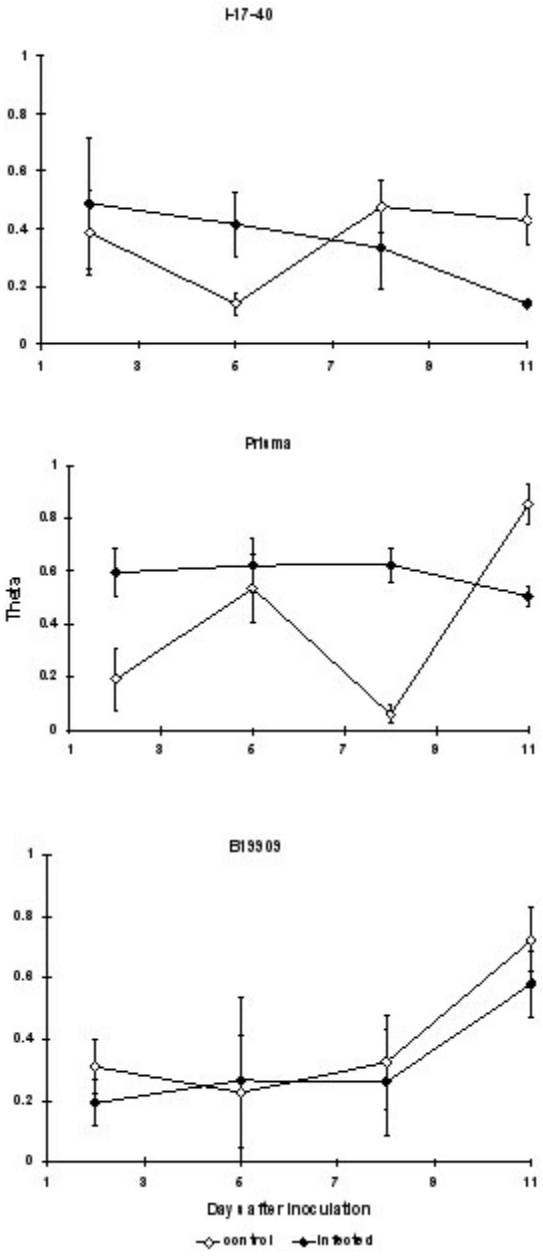

Fig. 3: The ratio of physical to total resistance to CO_2 diffusion in fourth leaves of three barley lines whose lower leaves were infected by mildew. Each datum point is the mean of three replicates

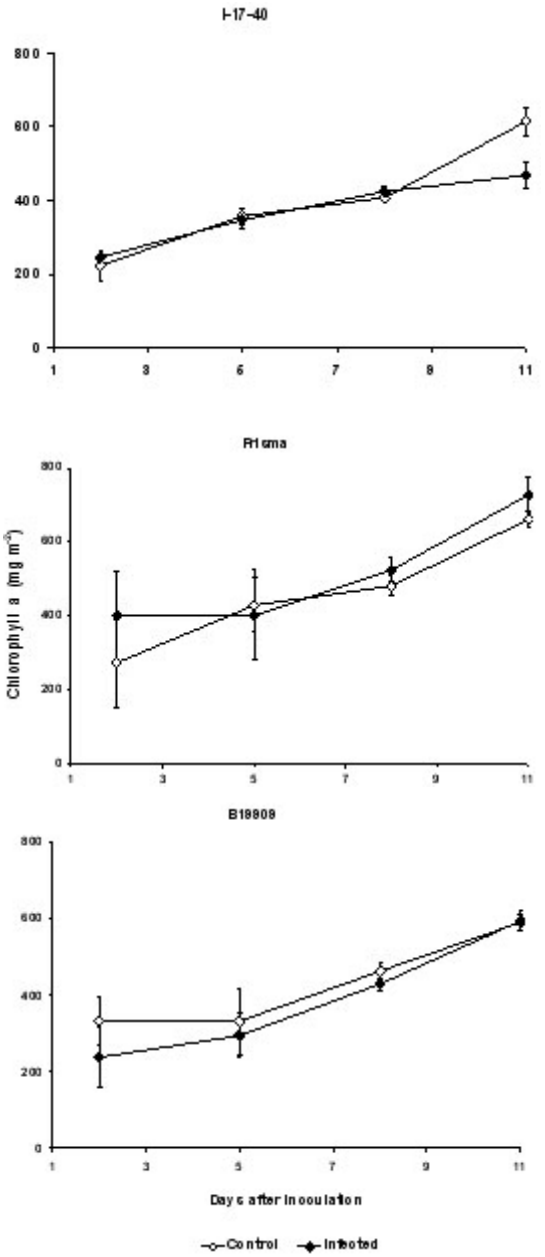

Fig. 4A: The chlorophyll a in fourth leaves of three barley lines with the lower three leaves infected by mildew. Each datum point is the mean of three replicates

Quantum efficiency or photochemical efficiency of photosynthesis (a)

The quantum efficiency of photosynthesis α of the three lines at each sampling time is plotted in Fig. 2. An analysis of variance showed that only in B19909, had α increased significantly ($p < 0.05$). In all other lines none of the differences was significant.

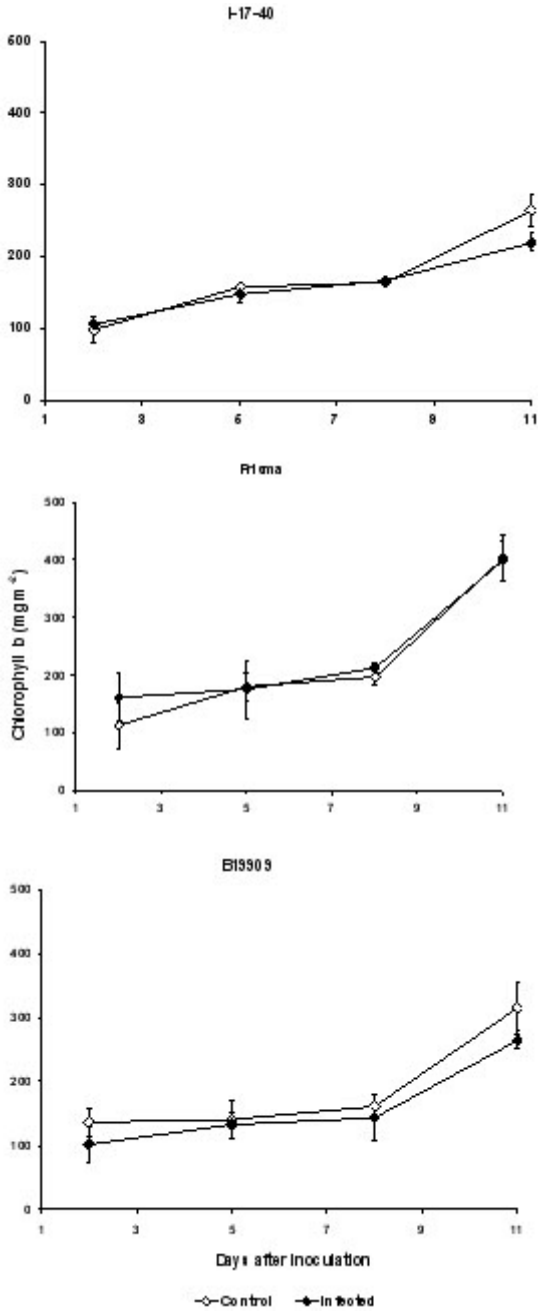

Fig. 4B: The chlorophyll b in fourth leaves of three barley lines with the lower three leaves infected by mildew. Each datum point is the mean of three replicates

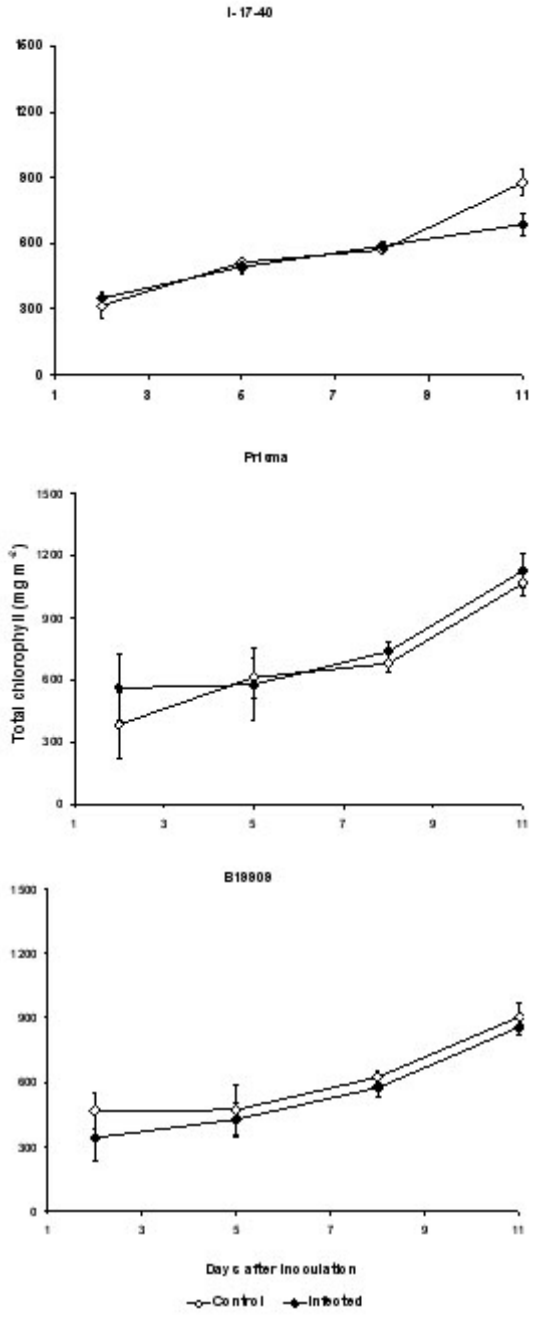

Fig. 4C: Total chlorophyll in fourth leaves of three barley lines with the lower three leaves infected by mildew. Each datum point is the mean of three replicates

The physical and biochemical resistance to CO_2 diffusion (q)

Changes in θ (a measure of the ratio of physical to total resistance to CO_2 diffusion into the leaf) in the uninfected fourth leaf of infected and uninfected plants are plotted in Fig. 3.

Infection of the lower three leaves increased θ in the uninfected fourth leaf of plants of both line I-17-40 and cv. Prisma up to the fifth and the eighth day after inoculation, respectively. Line B19909 showed fluctuating changes in θ to infection

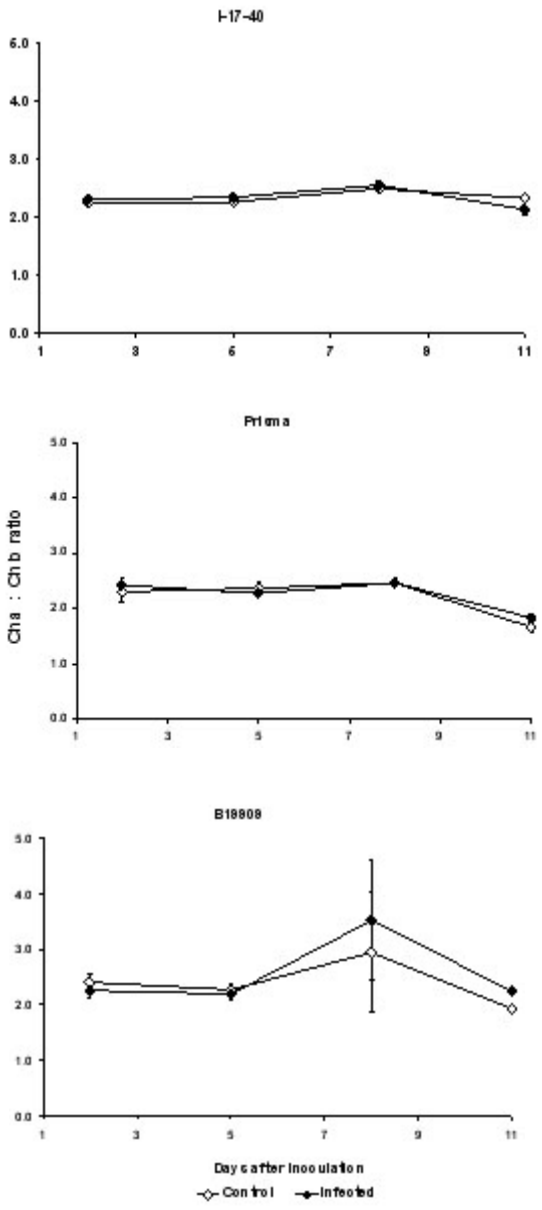

Fig. 4D: The chlorophyll a:b ratio in fourth leaves of three barley lines with the lower three leaves infected by mildew. Each datum point is the mean of three replicates

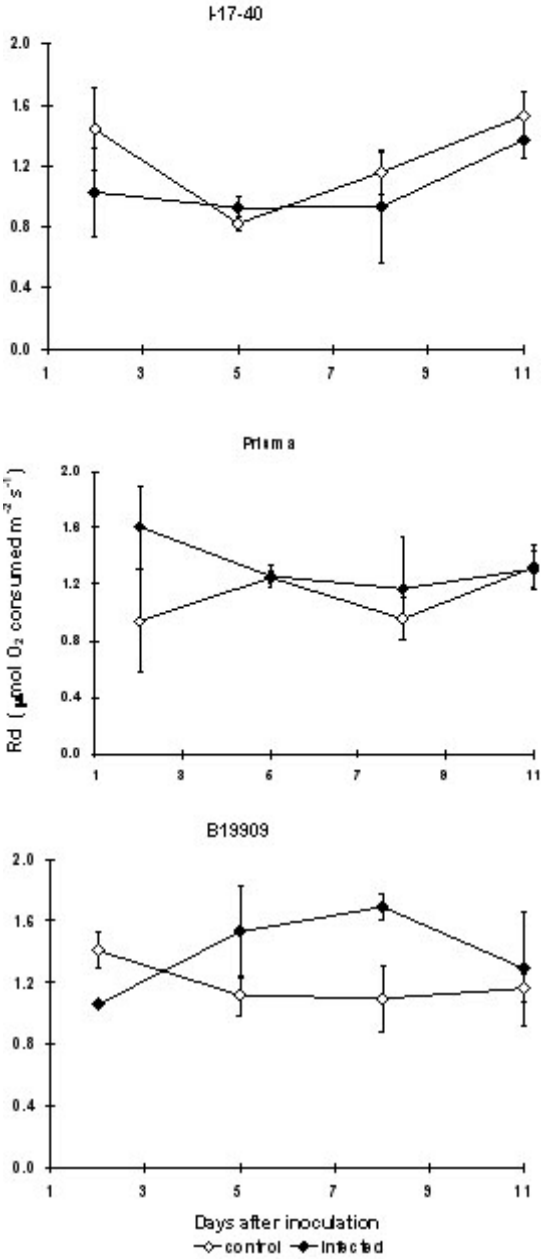

Fig. 5: Dark respiration per unit area in fourth leaves of three barley lines with lower three leaves infected by mildew. Each datum point is the mean of three replicates

of lower leaves between the second and the eighth day after inoculation, but none of the differences were significant. Although the analyses of variance showed that the differences were significant ($p < 0.05$) in both line I-17-40 and cv. Prisma but not in the wild line B19909, it is difficult to draw a conclusion because of the range in values found.

Chlorophyll content

Changes in chlorophyll content of the uninfected fourth leaves of infected and uninfected plants of each line, at each sampling time, are plotted graphically in Figs. 4A to 4D.

Fig. 4A shows that during the course of the experiment, chlorophyll a levels per unit leaf area in the uninfected fourth leaf of both infected and uninfected plants of all three lines increased. Infection did not significantly affect this increase except in the wild line I-17-40, which showed a significant reduction ($p < 0.05$) by the eleventh day following inoculation of the lower leaves.

The effects of infection on chlorophyll b levels (Fig. 4B) in the uninfected fourth leaf was generally similar to that of chlorophyll a (Fig. 4A). However, the slight increase in chlorophyll b observed in the fourth leaf of line I-17-40 eleven days after inoculation was not significant ($p > 0.05$). It follows that infection had no effects on total chlorophyll content (Fig. 4C) or on the chlorophyll a : b ratio (Fig. 4D).

Effects of infection on dark respiration

The rates of dark respiration in the uninfected fourth leaves of both infected and control plants of each line, are plotted in Fig. 5. Infection had no significant effect on dark respiration in the uninfected fourth leaf of infected plants compared to the controls in any of the three lines.

DISCUSSION

Many authors have shown that the rate of photosynthesis in uninfected parts of infected plants is stimulated by infection and clearly the more capacity a plant has for compensatory photosynthesis the higher the level of tolerance of infection it might possess. In the present study, infection of the three lower leaves was found to increase the rate of photosynthesis in an uninfected upper fourth leaf. The rates of maximum net and gross photosynthesis, expressed per unit leaf area, in the uninfected leaf on the infected plants were higher than in the corresponding leaf of uninfected plants in line B19909 but not in cv. Prisma and line I-17-40. The Quantum efficiency of photosynthesis (α) was also found to be slightly increased in the uninfected leaf of the infected plants of the wild barley B19909 but not in the other two lines. These increases may be attributed to an increase in light-harvesting capacity, a stimulation of photosynthetic electron transport, an increase in carboxylation efficiency, or a combination of these processes. When compared with controls, the uninfected fourth leaf of infected plants had similar chlorophyll levels and similar values of Pg_{max} and Pn_{max} expressed per unit chlorophyll. The ratios of physical resistance to total resistance to CO_2 diffusion into the leaf (θ) varied steadily between replicates but no significant differences were found between infected and uninfected plants in any line. Thus, the amount of CO_2 reaching the carboxylation sites appears not to be affected. Other mechanisms must be involved in the stimulation of photosynthesis in uninfected parts of infected plants in line B19909.

Although infection of the lower leaves gave a consistent slight increase in dark respiration in the uninfected fourth leaf in cv. Prisma and line B19909, none of the differences were significant. Thus, apart from the increase in the rate of $Pg_{max,}$ Pn_{max} and quantum efficiency in B19909, none of the parameters of photosynthesis measured were affected in any line.

These results are in line with those from previous studies, which have shown that powdery mildew infections of barley and also of pea stimulated photosynthesis in the upper, uninfected leaves of infected plants (Ayres, 1981b; Williams & Ayres, 1981; Walters & Ayres, 1983).

However, although line I-17-40 was shown in growth analysis experiments to compensate for the loss in vegetative growth caused in the early stages by mildew infection (Akhkha *et al.*, 2003a), no significant compensatory photosynthesis was observed in uninfected fourth leaf of infected plants of this line. This could be explained by the fact that

the mechanism or mechanisms responsible for compensatory photosynthesis are not activated until late in plant development.

The stimulation of net photosynthesis in uninfected leaves of mildewed barley plants was attributed, in part, to an increase in the amount and activity of RuBPcase (Walters & Ayres, 1983). An increase in the activities of phospho-enol-pyruvate carboxylase (PePcase) and NADP malic enzyme was also observed. Walters (1985) suggested that the changes in the nitrate / ammonium balance in infected shoots may have affected the activities of RuBPcase. He suggested also that the increased uptake of ^{32}P-labelled phosphate in mildewed barley could stimulate photosynthesis, either by increasing RuBPcase activity or by affecting the ratio of ATP / ADP.

The stimulation of photosynthesis in uninfected leaves of infected plants observed in wild barley B19909 and in other susceptible cultivars in other studies, may allow the plant to compensate for the loss of activity in the infected tissues and for the loss of photoassimilates to the pathogen. The increase in the compensatory photosynthesis in uninfected tissues of infected plants of the wild barley B19909 must play at least some part in compensating for plant growth losses to the pathogen. Thus compensatory activity may play a role in tolerance.

REFERENCES

1. Akhkha, A; Clark, D. D. & Dominy, P.J. Relative tolerances of wild and cultivated barley to infection by *Blumeria graminis f.sp. hordei* (Syn. *Erysiphe graminis f.sp. hordei*). I-the effects of infection on growth and development. *Physiological & Molecular Plant Pathology* **62**(4): 237-250 (2003a).
2. Akhkha, A; Clark, D. D. & Dominy, P. J. Relative tolerances of wild and cultivated barley to infection by *Blumeria graminis f.sp. hordei* (Syn. *Erysiphe graminis f.sp. hordei*). II-the effects of infection on photosynthesis and respiration. *Physiological & Molecular Plant Pathology* **62**(6): 347-354 (2003b).
3. Akhkha, A; Reid, I.; Clark, D. D. & Dominy, P. J., Photosynthetic light response curves determined with the leaf oxygen electrode: minimisation of errors and significance of the convexity term. *Planta* **214**(1): 135-141 (2001).
4. Arnon, D. I., Copper enzymes in isolated chloroplasts. Polyphenol oxidases in *Beta vulgaris*. *Plant Physiology* **24**: 1-14 (1949).
5. Ayres, P. G., Powdery mildew stimulated photosynthesis in uninfected leaves of pea plants. *Phytopathologische Zeitschrift* **100**: 312-318 (1981).
6. Delieu, T. & Walker, D. A., Polarographic measurement of photosynthetic O2 evolution by leaf discs. *New Phytologist* **89**: 165-175 (1981).
7. Delieu, T. & Walker, D. A., Simultaneous measurement of O_2-evolution and chlorophyll fluorescence from leaf pieces. *Plant Physiology* **73**: 534-541 (1983).
8. Hipkins, M. F. & Baker, N. R., Photosynthesis Energy transduction, a practical approach. pp. 62-64. IRL Press (1986).
9. Holden, M., Chlorophylls. In: Chemistry and Biochemistry of Plant Pigments. Ed. by Goodwin, T. W. pp. 461-485. Academic Press. London and New York (1965).
10. Livne, A., Photosynthesis in healthy and rust affected plants. *Plant Physiology* **39**: 614-621 (1964).
11. Mackinney, G., Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry* **140**: 315-32 (1941).
12. Sabri, N., A study of the effects of powdery mildew *Erysiphe graminis* f.sp. *avenae*, on the growth and development of wild and cultivated oats. *Ph.D. Thesis*. Botany Department. University of Glasgow (1993).
13. Walters, D. R. & Ayres, P. G., Changes in nitrogen utilisation and enzyme activities associated with CO_2 exchanges in healthy

leaves of powdery mildew infected barley. *Physiological Plant Pathology* **23**: 447-459 (1983).

14. Walters, D. R., Shoot : Root interrelationships The effects of obligately biotrophic fungal pathogens. *Biological Review* **60**: 47-79 (1985).

15. Williams, G. M. & Ayres, P. G., Effects of powdery mildew and water stress on CO_2 exchanges in uninfected leaves of barley plants. *Plant Physiology* **68**: 527-530 (1981).