Nucleoside/nucleotide pools in wheat leaves naturally infected with Erysiphe graminis. I. Changes in the flag leaf

ARTICLE *in* PHYSIOLOGICAL AND MOLECULAR PLANT PATHOLOGY · NOVEMBER 1988

Impact Factor: 1.41 · DOI: 10.1016/0885-5765(88)90015-X

Physiological and Molecular Plant Pathology (1988) **33,** 497–506

Nucleoside/nucleotide pools in wheat leaves naturally infected with *Erysiphe graminis*. I. Changes in the flag leaf

A. SAWERT, H.-J. AUST† and K. G. WAGNER‡

Gesellschaft für Biotechnologische Forschung, Ag. Enzymologie, Mascheroder Weg 1, D-3300 Braunschweig and †Institut für Mikrobiologie, Ag. Mykologie und Phykologie, Technische Universität Braunschweig, F.R.G.

(*Accepted for publication June 1988*)

Nucleoside and nucleotide contents of the flag leaves of wheat plants, naturally infected with the powdery mildew fungus *Erysiphe graminis* were analysed in a field experiment. Fungicide treated plants were used as controls. Only low levels of infection occurred during the experiment (1% to 4% of the leaf area), but they resulted in higher leaf dry weight, and lower levels of chlorophyll, inorganic phosphate and NADP compared with the control plants. Although the total adenine nucleotide pools were the same in the flag leaves of infected and control plants, the energy charge values were slightly larger in the infected flag leaves. The total nucleoside content was higher in the infected flag leaves than the control; while the adenosine pool decreased, that of uridine increased strongly. Although the level of UDP-glucose was similar in the flag leaves of infected and control plants only decreasing slightly with time, UDP-N-acetylglucosamine, a precursor for fungal chitin biosynthesis, showed very different behaviour. The amounts of UDP-N-acetylglucosamine were very low in the control plants but increased greatly in the infected flag leaves to very high values (900 nmol g^{-1} d.wt) obviously reflecting exploitation of the hosts metabolism by the fungus for precursors for chitin biosynthesis. The size of the uridine pools was also correlated with the degree of infection and probably reflected recycling of the UDP moiety.

INTRODUCTION

The interaction between biotrophic fungal plant pathogens and their hosts metabolism is an interesting area of research and although, in general, knowledge is limited, effects on photosynthesis, respiration and translocation have been studied in some detail [*4, 8, 9, 11–15, 17, 18, 27, 29*].

Synthesis and consumption of nucleotides are essential elements of the primary metabolism of a plant with changes affecting not only energy metabolism but also various aspects of anabolism. Infections by biotrophic parasites would be expected to interfere with the nucleotide metabolism of the host and knowing what these changes are might help to elucidate the nature of the dependence of the biotrophic parasite on its host's metabolism. Using enzymatic methods, adenine nucleotide [*16*] and pyridine nucleotide pools [*23*] have been determined in wheat infected by *Puccinia graminis* (black stem rust) and in barley infected by *Erysiphe graminis* and the application of the more powerful HPLC techniques [*3, 19, 21, 24*] enables a larger range of nucleotides and

‡To whom correspondence should be addressed.

Abbreviations used in text: Ado, adenosine; Guo, guanosine, Urd, uridine; UDP-Glc, UDP-glucose; UDP-GlcNAc, UDP-N-acetylglucosamine.

0885–5765/88/060497 + 10 $03.00/0

nucleosides to be assayed. Previous work has shown that cereals have very high precursor pools of nucleosides and adenine, indicating possible differences relative to dicotyledons in their enzyme equipment for the salvage pathways of nucleobase catabolism [*24, 25*]. Knowledge of the size of these pools provides additional information on changes in nucleotide precursor production or consumption brought about by parasite interference. Furthermore, recent analyses on *Puccinia striiformis* infected young wheat plants [*5, 6*] have indicated that monitoring nucleoside/nucleotide pools may provide a useful measure of the status of the pathogen–host interaction. In the earlier study we used reverse-phase HPLC only and thus could not determine nucleoside diphosphate sugar pools. The present work analyses changes in precursor and nucleotide pools in wheat plants naturally infected with the powdery mildew fungus *Erysiphe graminis*. The application of both reverse-phase and anion-exchange HPLC, enabled a broad spectrum of nucleotides, including the precursor for fungal chitin biosynthesis, UDP-N-acetylglucosamine, to be determined.

MATERIALS AND METHODS

Growth of plants

Wheat plants, *Triticum aestivum* L. cv. Kanzler were autumn sown in the field of the Biologische Bundesanstalt Braunschweig in 1984. The experimental plot (12 × 8 m) was untreated, whereas the control (a plot of about 100 m^2 integrated into a larger area of the field) was protected throughout the season with fungicides as indicated below. The distance between the two plots was only 22 m, and so apart from the fungicide treatment all conditions should have been essentially the same. Infections in the untreated plots were mainly powdery mildew with other pathogens rarely evident except during the later stages of growth when brown rust developed to a maximum level of infection of about 0·5%. The degree of mildew infection was determined at each harvest from 30 plant samples. Geeen leaf area was determined with a planimeter (after removing the clorotic parts) and the total area of mildew pustules was estimated by totalling the areas covered by all colonies.

Fungicide treatments of the control plot were carried out as follows: 17 May, 0·3 kg ha^{-1} Derosal (Carbendazim, Hoechst AG), 20 May, 0·5 l ha^{-1} Desmel (Propiconazol, Ciba Geigy), 3 June, 1 l ha^{-1} Corbel (Fenpropimorph, BASF), 19 June, 2·5 kg ha^{-1} Tilt (Captafol/Halacrinat, Ciba Geigy), 24 June, 1 l ha^{-1} Corbel, and 27 June, 0·5 kg ha^{-1} Bayleton (Triadimephon, Bayer A.G.). The flag leaves were sampled on 19 June before the fungicide treatment of that day was applied.

Biochemical determinations

Nucleoside/nucleotide determinations were performed on the flag leaves from 10 plants at each harvest. The plants were harvested at noon, their flag leaves pooled and immediately frozen in liquid nitrogen. The leaves were extracted and the extracts purified for HPLC analysis as described earlier [*19, 24*]. The leaves were extracted twice, and each extract was analysed once or twice by HPLC. Chlorophyll was determined using the method of Arnon [*2*]. Inorganic phosphate was determined in 100 µl fractions of the original extract [*19*] which had been frozen and stored at −70 °C. Phosphate was

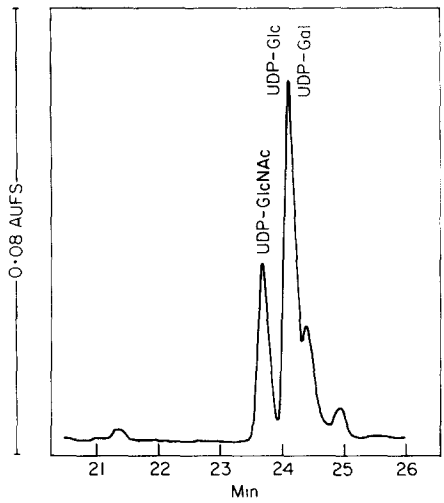

FIG. 1. Separation of UDP-sugars by anion-exchange HPLC. Only the trace relating to the UDP-sugars is shown (for the full chromatogram see [*19*]). 5 mM NaH_2PO_4 at pH 3·0 (buffer A) and 500 mM NaH_2PO_4 at pH 4·0 (buffer B) were used. The elution program was started with buffer A (5 min isocratic) followed by a linear gradient from buffer A to B (50 min) and 10 min buffer B (isocratic). The column was run at ambient temperature with a flow rate of 1 ml min^{-1}; absorbance measured at 254 nm with 0·08 absorption units at full scale (AUFS).

assayed according to the method of Eibl & Lands [*10*] using reagents obtained from Serva Feinbiochemica, Heidelberg.

HPLC analysis

A 5 μm Shandon APS-Hypersil column (250 × 4·6 mm) from Melz, Berlin was used. The conditions for anion-exchange chromatography [*19*] were modified in order to improve the resolution of the UDP-sugars with potassium phosphate buffer being replaced by sodium phosphate. This resulted in improved base line stability. The concentrations used and gradient conditions were as indicated in the legend to Fig. 1.

The reverse-phase column was used to determine all ribonucleosides and adenine containing ribonucleotides including NAD and NADP, while the other ribonucleotides and nucleoside diphosphate sugars were determined by anion-exchange HPLC. Recovery of individual nucleotides was more than 80%, recovery experiments being performed using 2 nmol (NADP 1 nmol) of authentic reference compounds [*19, 24*]. Furthermore, on-line UV spectroscopy (2140 rapid spectral detector from LKB, Bromma Sweden) was used to confirm the purity and the identity of the individual peaks [*19, 24*].

Nucleoside diphosphate sugars were resolved by anion-exchange HPLC which separated them according to the number of negative charges possessed and according to the nucleobase. A cluster of peaks containing neutral sugars and UDP appeared between the nucleoside monophosphates and diphosphates [*19*] and their resolution was significantly improved by using the sodium phosphate buffer and the Shandon APS Hypersil

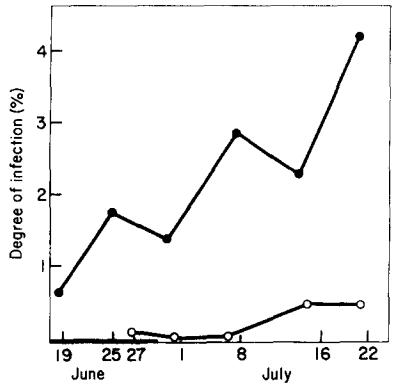

FIG. 2. *Erysiphe graminis* infection of the flag leaves of unprotected wheat plants (●) and fungicide protected control plants (○). Degree of infection is given as percentage of flag leaf area covered by mildew pustules (see Materials and Methods).

column (Fig. 1). The UDP-N-acetylglucosamine (UDP-GlcNAc) peak was well separated from UDP-Glc and UDP-Gal while a fourth, minor peak, also with a UDP moiety, remains to be characterized.

RESULTS

Development of mildew infection, dry weight and chlorophyll content of the flag leaves

The field grown wheat plants had their leaves fully developed and were just beginning to flower at the first harvest on June 19. Figure 2 shows the development of mildew infection on the flag leaves of infected and control plants. The degree of infection of the infected plants, indicated as percentage of leaf area covered by mildew pustules, ranged from 1% at the first harvest to about 4% at the final harvest. Control plants developed slight infections only up to about 0·5% by the final harvest. Thus the results presented here are from flag leaves with relatively low degrees of infection. However, the degree of infection on the lower leaves of the experimental plants was higher (see accompanying paper [*26*]), up to 8% on the fourth leaf of the first harvest.

The measurements of chlorophyll content [Fig. 3(a)], show that senescence in the control plant flag leaves only commenced at the very end of the experiment, whereas in the mildew infected plants it occurred about 14 days earlier and was quite marked in the later stages. The dry weight of the flag leaves [Fig. 3(b)] was about 30% of the fresh weight and thus considerably higher than was found in the leaves of the younger greenhouse-grown wheat plants studied previously [*5*]. The percentage dry weight remained fairly constant in the control leaves throughout the experiment, whereas the infected leaves showed an increase at the later stages. This increase probably reflects loss of water due to increased transpiration from the infected tissues and loss of stomatal control and/or accumulation of inorganic or organic compounds perhaps due to inhibition of remobilization.

The inorganic phosphate content of the flag leaves of control plants and infected plants [Fig. 3(c)] decreased during the course of the experiment but to much lower levels

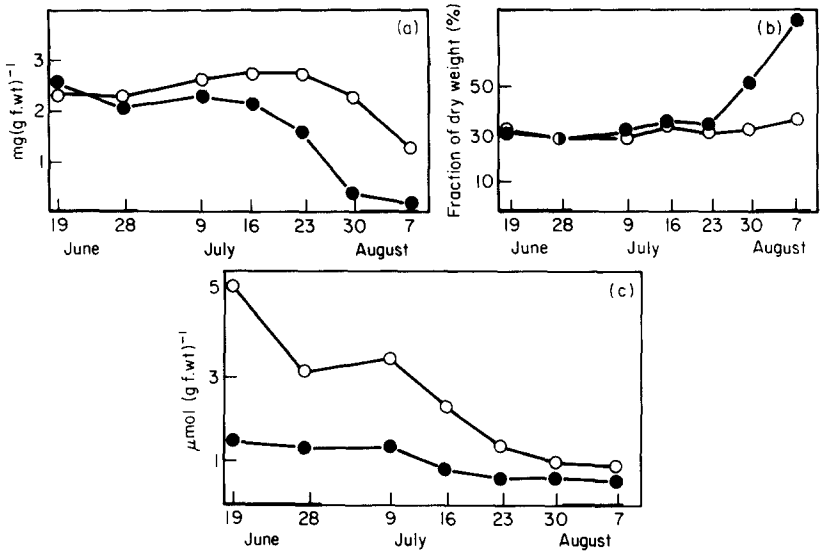

FIG. 3. (a) Chlorophyll content, (b) relative dry weight and (c) inorganic phosphate content of the flag leaves of *Erysiphe graminis* infected (●) and control (○) wheat plants. Standard deviations of chlorophyll determinations were all less than ± 10%. Fungicide treatments were applied on 19 June (Tilt), 24 June (Corbel) and 27 June (Bayleton) (see Materials and Methods). The 19 June samples were taken before the fungicide treatment.

in the infected plants. The large difference in levels even at the beginning of the experiment, when the levels of infection on the flag leaf were quite low, is astonishing. Fungicide treatment of the control plants could have been a possible reason. However, as reported in the following paper [*26*] the levels of infection in the lower, older leaves were significant at this stage and so reduced levels in the flag leaf could be a consequence of increased strength of phosphate sinks [*1*, *22*] in the lower leaves.

Nucleotide contents of flag leaves of control and infected plants

The main nucleotide pools were those of ATP and ADP followed by the pyridine nucleotides, the UDP-sugars and UTP. The levels of guanine nucleotides and CTP were markedly lower. In contrast to green-house grown wheat plants [*24*, *25*], the flag leaves contained significant but small pools of nucleosides with uridine as the main component. A comparison of the flag leaf pools from infected and control plants showed considerable differences, especially in the pools of UDP-GlcNAc and uridine which were much higher in the infected plants.

There were no differences between control and infected plants in their total adenine nucleotide pools until the last two harvests when the reductions paralled the differences in the rates of senescence (Fig. 4). The greater reductions in the infected leaves was obviously related to the increased percentage dry weights [Fig. 3(b)] since the nucleotide pools were related to the dry weight. The energy charge values, however, derived from the adenine nucleotide pools were slightly higher in the infected plants.

Changes in NAD pools in the flag leaf (Fig. 5) were similar in both infected and control plants but reflected the development of senescence (Fig. 3) more closely than the

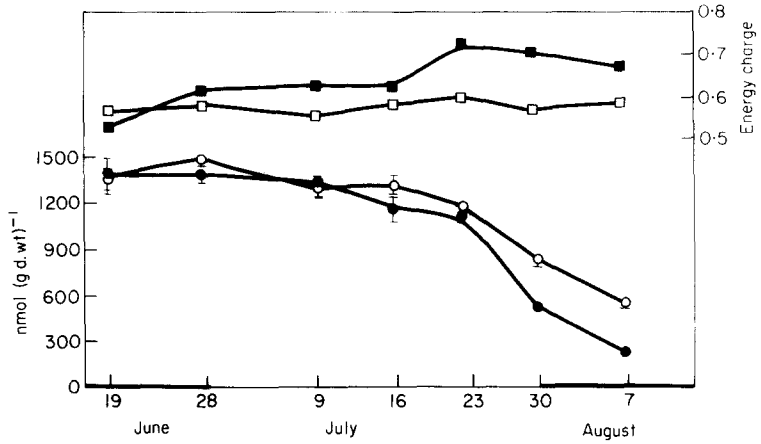

FIG. 4. Total adenine nucleotides (ATP, ADP, AMP) (○, ●) and adenine nucleotide derived energy charge (ATP + 1/2 ADP)/(ATP + ADP + AMP) (□, ■) of flag leaves from infected (●, ■) and control plants (○, □). Standard deviations for the energy charge values were all $< \pm 0{\cdot}015$ and thus within the limits of the symbol. The standard deviations of the total adenine nucleotide determinations are given except where they were within the limits of the symbol.

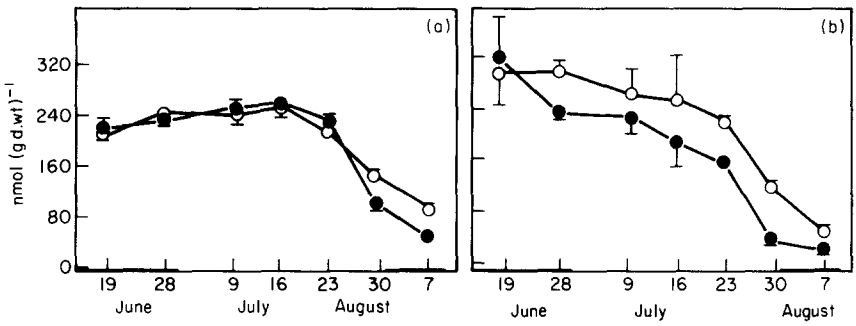

FIG. 5. Time course of changes in (a) NAD and (b) NADP pools in infected (●) and control (○) flag leaves. Standard deviations are marked on one side of the points only.

adenine nucleotides did (Fig. 4). The NADP pools showed a similar time course, with the infected leaves having slightly lower levels. The lower NADP pools and chlorophyll content [Fig. 3(a)] are probably associated with the reduced photosynthetic activity of the infected tissue.

Nucleosides and UDP-sugars

The most significant differences in the nucleoside pools between infected and control flag leaves were observed in relation to uridine levels (Fig. 6). Whereas the adenosine content decreased during leaf senescence, with infected and control leaves behaving similarly, the uridine pools were low from the start and remained so in the control leaves but increased rapidly in the infected flag leaves during the later stages of the experiment.

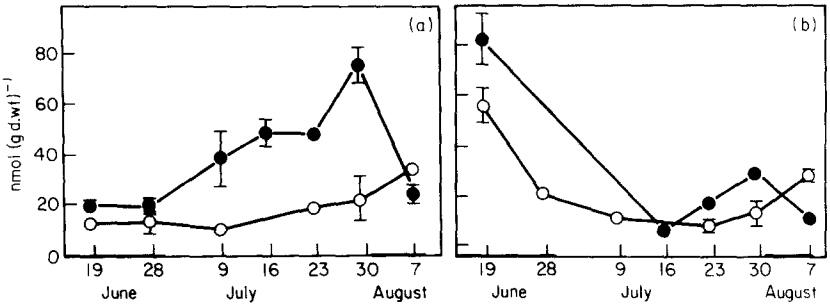

FIG. 6. (a) Uridine and (b) adenosine pools in infected (●) and control (○) flag leaves. The adenosine pool in the controls on 28 June and 9 July were not determined due to contamination of the peaks with an impurity.

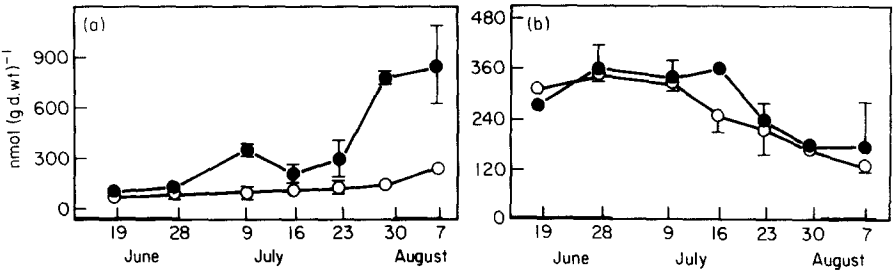

FIG. 7. (a) UDP-N-acetylglycosamine and (b) UDP-glucose pools in infected (●) and control (○) flag leaves.

Guanosine content was low, below 20 nmol (g d.wt)$^{-1}$ and only increased slightly in the infected leaves during the course of the experiment (data not shown) thus showing a similar trend to uridine.

In uninfected leaves, UDP-Glc was the main component of the nucleoside diphosphate sugars [Fig. 7(b)], where its concentration amounted to almost 400 nmol (g d.wt)$^{-1}$. The time course changes reflected leaf senescence with no major differences being found between control and infected leaves. The concentration of UDP-GlcNAc, a precursor for protein glycosylation in plant metabolism [7] and for fungal chitin biosynthesis, was rather low and constant in the control plants but in the infected flag leaves, it had increased to 900 nmol (g d.wt)$^{-1}$ by the end of the experiment. This indicates a mobilization of plant metabolism in the direction of fungal chitin biosynthesis.

DISCUSSION

The present results were obtained from analyses of the flag leaves in a field experiment in which only a low level of mildew infection (1%) was present at the beginning of the experiment and in which the level of infection reached 4% at the end of the experiment.

However, it must be remembered that the lower leaves showed high levels of infection, as reported in the following paper [*26*].

The control plants were protected by fungicides. The ideal control would have been non-sprayed uninfected plants but unfortunately this is not possible with a field experiment. The fungicide treatments themselves could affect nucleotide pools in the plant, although little is known on this subject. However, it is perhaps relevant to note that several nucleoside/nucleotide pools were found to be very similar between fungicide treated and infected plants during the course of the experiment indicating that either the infection and fungicide treatments had similar effects on the plants or that neither did.

The present findings of lower free phosphate pools in infected than in the fungicide-protected control plants is probably due to the conversion of free phosphate by the fungus in the infected leaves into polyphosphates. Walters & Ayres [*28*] reported both higher rates of uptake and increased amounts of phosphate in barley roots due to powdery mildew infection, and also a higher degree of translocation to the shoots. This could also be explained by a higher sink activity in the infected leaves. In this respect it is interesting that *P. striiformis* infected young greenhouse-grown wheat plants [*5*] had higher free phosphate levels in the infected areas of leaves relative to the healthy parts of the same leaf (A. Backer, unpublished results) (cf. also [*29*]). This indicates differences between the effects of yellow rust and powdery mildew infections. However, whether the reduction in the export rate of phosphate from the first leaves into the phloem observed in *P. recondita* infected barley plants [*11*] is connected to these findings is at present a matter of speculation.

The results from the control plants, and also in part from the infected plants, give some insight into leaf senescence. The time course changes in the important pools, such as the pyridine nucleotides, UDP-glucose, adenine nucleotides and adenosine, and also in chlorophyll contents clearly reflect leaf senescence. Interestingly the energy charge did not decline, indicating that this value is maintained at a high level even in senescing leaves. This confirms data obtained from uninfected wheat and barley leaves [*25*] and also from tobacco plants [*20*].

Infection may promote senescence. This was shown by the reduction in chlorophyll levels and in the NADP pools, although certain pools of other nucleotides were not affected. Powdery mildew infections have been shown to increase respiratory activity and this increased activity has been shown to be mainly due to host tissue [*27*]. Changes in respiratory activity are indicated by the present results, for example the time course changes in NAD and NADP pools (Fig. 5). The NADP content markedly decreased in the infected flag leaves and although we have not determined the NADPH content, the reduced NADP levels could reflect reduced photosynthetic activity as has been reported for infected tissue [*8*]. In contrast the NAD content was much the same in infected as in control flag leaves at all harvests except the last two indicating that the respiratory activity of the infected leaves was little affected during the early phases of the infection. The calculated NAD/NADP ratios indicate a rather constant value of 0·8 in control leaves which increased at later stages to about 2 (7 August). In infected leaves the NAD/NADP ratios were slightly larger than those of the control in the earlier phase and increased to a value of about 4 on 7 August.

We have recently shown in *P. striiformis* infected wheat [*5, 6*], that infection increased not only the NAD/NADP ratio but also the total adenine nucleotide pool and the energy

charge. Hoppe & Heitefuss [*16*] also observed a large increase in the adenine nucleotide pools in *P. graminis* infected wheat, but in contrast found that the energy charge decreased slightly with increased development of the rust. As these authors observed large increases in the ATP/ADP ratio, the decreased energy charge values observed could result from problems associated with enzymatic determination of AMP. In contrast to infections with *P. striiformis* [*5, 6*], the present data from powdery mildew did not reveal any increase in total adenine nucleotide pools, although the energy charge increased slightly in the later stages.

The most interesting findings of these experiments are that certain pools such as UPD-GlcNAc and uridine show a close correlation with the degree of infection. The former pool provides the activated building units for fungal chitin biosynthesis, whereas the plant utilizes it for glycoprotein synthesis [*7*]. The large increase in the infected leaves over the basal levels in the control plants indicates overproduction of this metabolite for fungal usage. After incorporation of N-acetylglucosamine into chitin, UDP is liberated and assuming that the UDP is transformed to uridine, the high pool of the latter and its correlation with infection could indicate a recycling process. The following paper [*26*] provides a more detailed examination of UDP-GlcNAc formation, utilization and UDP recycling.

We are grateful to Mr U. Hirschgänger and Mr H. Könning for the determination of the degree of infection, to Dr V. Wray for linguistic advice, to Mrs H. Starke for typing the manuscript and to Mrs C. Lippelt for preparing the graphs. Special thanks are also due to Prof. G. Bartels from the Biologische Bundesanstalt Braunschweig. This work has been supported by the Deutsche Forschungsgemeinschaft (Wa 91) and the Fonds der Chemischen Industrie.

REFERENCES

1. Ahmad, I., Owera, S. A. P., Farrar, J. F. & Whitbread, R. (1982). The distribution of five major nutrients in barley plants infected with brown rust. *Physiological Plant Pathology* **21,** 335–346.
2. Arnon, D. J. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaries*. *Plant Physiology* **24,** 1–15.
3. Ashihara, H., Mitsui, K. & Ukaji, T. (1987). A simple analysis of purine and pyrimidine nucleotides in plant cells by high-performance liquid chromatography. *Zeitschrift für Naturforschung* **42c,** 297–299.
4. Ayres, P. G. (1984). Effects of infection on root growth and function; consequences for plant nutrient and water relations. In: Plant Diseases: Infection, Damage and Loss, Ed. by R. K. S. Wood & G. J. Jellis, pp. 105–117. Blackwell Scientific Publications, Oxford.
5. Backer, A., Sawert, A., Aust, H.-J. & Wagner, K. G. (1988). Nucleoside–nucleotide pools in yellow rust infected wheat. *Physiological Molecular Plant Pathology* **32,** 467–476.
6. Backer, A., Sawert, A., Aust, H.-J. & Wagner, K. G. (1988). Diurnal changes of nucleoside/nucleotide pools in healthy and yellow rust infected wheat plants. *Physiological Molecular Plant Pathology* **32,** 477–481.
7. Beevers, L. (1982). Amino sugars—plants and fungi. In *Encyclopedia of Plant Physiology*, New Series, Vol. 13A, *Plant Carbohydrates I: Intracellular Carbohydrates*, Ed. by F. A. Loewus & W. Tanner, pp. 103–123. Springer-Verlag, Berlin.
8. Buchanan, B. B., Hutcheson, S. W., Magyarosy, A. C. & Montalbini, P. (1981). Photosynthesis in healthy and diseased plants. In *Effects of Disease on the Physiology of the Growing Plant*, Ed. by P. G. Ayres, pp. 13–28. Society for Experimental Biology, Seminar Series II. Cambridge University Press, Cambridge.
9. Chakravorty, A. K. & Scott, K. J. (1982). Biochemistry of host rust interaction. In *The Rust Fungi*, Ed. by K. J. Scott & A. K. Chakravorty, pp. 180–222. Academic Press, London.
10. Eibl, H. & Lands, W. E. M. (1969). A new, sensitive determination of phosphate. *Analytical Biochemistry* **30,** 51–57.
11. Farrar, J. F. (1984). Effects of pathogens on plant transport systems. In *Plant Diseases: Infection, Damage and Loss*, Ed. by R. K. S. Wood & G. J. Jellis, pp. 87–104. Blackwell Scientific Publications, Oxford.

12. Farrar, J. F. (1985). Carbohydrate metabolism in biotrophic plant pathogens. *Microbiological Sciences* **2,** 314–317.
13. Gay, J. L. (1984). Mechanisms of biotrophy in fungal pathogens. In *Plant Diseases: Infection, Damage and Loss*. R. K. S. Wood & G. J. Jellis, pp. 49–59. Blackwell Scientific Publications, Oxford.
14. Gay, J. L. & Manners, J. M. (1981). Transport of host assimilates to the pathogen. In *Effects of Disease on the Physiology of the Growing Plant*; Ed. by P. G. Ayres, pp. 85–100. Society for Experimental Biology, Seminar Series II. Cambridge University Press, Cambridge.
15. Habeshaw, D. (1984). Effects of pathogens on photosynthesis. In *Plant Diseases: Infection, Damage and Loss*, Ed. by R. K. S. Wood & G. J. Jellis, pp. 63–72. Blackwell Scientific Publications, Oxford.
16. Hoppe, J. H. & Heitefuss, R. (1976). Untersuchungen zur Regulation des Kohlenhydratstoffwechsels in Weizenpflanzen nach Infektion mit *Puccinia graminis tritici*. *Phytopathologische Zeitschrift* **86,** 37–55.
17. Kosuge, T. & Kimpel, J. A. (1981). Energy use and metabolic regulation in plant-pathogen interactions. In *Effects of Disease on the Physiology of the Growing Plant*, Ed. by P. G. Ayres, pp. 29–45. Society for Experimental Biology, Seminar Series II. Cambridge University Press, Cambridge.
18. Manners, J. M., Gay, J. L. (1983). The host-parasite interface and nutrient transfer in biotrophic parasitism. In *Biochemical Plant Pathology*, J. A. Callow, pp. 163–195. John Wiley, Chichester.
19. Meyer, R. & Wagner, K. G. (1985). Determination of nucleotide pools in plant tissue by high-performance liquid chromatography. *Analytical Biochemistry* **148,** 269–276.
20. Meyer, R. & Wagner, K. G. (1986). Nucleotide pools in leaf and root tissue of tobacco plants: influence of leaf senescence. *Physiologica Plantarum* **67,** 666–672.
21. Nieman, R. H. & Clark, R. A. (1984). Measurement of plant nucleotides by high-performance liquid chromatography. *Journal of Chromatography* **317,** 271–281.
22. Pozsar, B. I. & Kiraly, Z. (1966). Phloem-transport in rust infected plants and the cytokinin-directed long-distance movement of nutrients. *Phytopathologische Zeitschrift* **56,** 297–309.
23. Ryrie, I. J. & Scott, K. J. (1968). Metabolic regulation in diseased leaves II. Changes in nicotinamide nucleotide co-enzymes in barley leaves infected with powdery mildew. *Plant Physiology* **43,** 687–692.
24. Sawert, A., Backer, A., Plank-Schumacher, K. & Wagner, K. G. (1987). Determination of nucleotides and nucleosides in cereal leaves by high performance liquid chromatography. *Journal of Plant Physiology* **127,** 183–186.
25. Sawert, A., Backer, A. & Wagner, K. G. (1988). Nucleoside pools in cereal leaves strongly depend on leaf age. *Plant Cell Physiology* **29,** 61–65.
26. Sawert, A., Aust, M.-J. & Wagner, K. G. (1988). Nucleoside/nucleotide pools of wheat leaves infected with powdery mildew II. Effects of leaf age and intensity of infection. *Physiological Molecular Plant Pathology* **33,** 453–460.
27. Smedegaard-Petersen, V. (1984). The role of respiration and energy generation in diseased and disease-resistant plants. In *Plant Diseases: Infection, Damage and Loss*, Ed. by R. K. S. Wood & G. J. Jellis, pp. 73–85. Blackwell Scientific Publications, Oxford.
28. Walters, D. R. & Ayres, P. G. (1981). Phosphate uptake and translocation by roots of powdery mildew infected barley. *Physiological Plant Pathology* **18,** 195–205.
29. Whipps, J. M. & Lewis, D. H. (1981). Patterns of translocation, storage and interconversion of carbohydrates. In *Effects of Disease on the Physiology of the Growing Plant*, Ed. by P. G. Ayres, pp. 47–83. Society for Experimental Biology, Seminar Series II. Cambridge University Press, Cambridge.