Effects of Powdery Mildew Infection of Barley on the Ascorbate-Glutathione Cycle and Other Antioxidants in Different Host-Pathogen Interactions

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ABSTRACT

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Rate of lipid peroxidation (malondialdehyde formation), levels of ascorbic acid and nonprotein thiols, and activities of ascorbate peroxidase (AP), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione S-transferase (GST), and quinone reductase (QR) were determined in leaves of three barley cultivars inoculated by a Hungarian isolate of *Erysiphe graminis* f. sp. *hordei*. Markedly increased malondialdehyde levels (enhanced lipid peroxidation) were observed in leaves of the resistant cultivar Amsel after infection but not in two susceptible cultivars. In the diseased susceptible cultivars Emir and GK-Omega, however, the ascorbic acid levels substantially decreased. A substantial increase of AP and a decline of DHAR activities also were observed in mildewed susceptible plants. A dramatic induction of NADPH-consuming activity was found in the inoculated leaves of the highly susceptible cultivar Emir concomitantly with decreasing 1-electron QR activity. Less-pronounced changes in the parameters were found in the resistant cultivar Amsel. Thiol levels increased moderately in cultivar Amsel and in susceptible cultivar GK-Omega. No significant change in GR activity was found in either cultivar. GST activity was induced in each inoculated cultivar, most substantially in highly susceptible Emir (up to about 360% of the control). Several antioxidative processes seemed to be activated in compatible host-parasite relationships, which may diminish the damaging effects of oxidative stress. This supposition was confirmed by infecting one barley cultivar (Amsel) with compatible and incompatible mildew races. These antioxidative processes were less efficiently activated in the incompatible relationship, which may lead to an early necrotization in the resistant host.

Additional keywords: *Hordeum vulgare*.

The increased production of reduced, active derivatives of oxygen (oxidative stress) is associated with the plant defense response in various plant-pathogen interactions (20,24,35,36), including powdery mildew infection (22). Active oxygen species also are formed during normal plant metabolism, and plant cells contain efficient antioxidative defense systems to counteract the toxicity of these species (10). A principal antioxidative system is the ascorbate-glutathione (GSH) cycle, which detoxifies hydrogen peroxide in chloroplasts (Fig. 1). In this system, the reduction of H_2O_2 is achieved at the expense of the photosynthetically produced NADPH, using GSH, ascorbate peroxidase (AP, EC 1.11.1.11), dehydroascorbate reductase (DHAR, EC 1.8.5.1), and glutathione reductase (GR, EC 1.6.4.2) for the regeneration of the primary antioxidant ascorbic acid (10,26). The function of this cycle is strongly influenced by a wide range of abiotic stress effects. Increased ascorbate levels (28,33), GSH contents (34), and elevated AP (12,25), DHAR (30), and GR activities (8) have been detected in different plant tissues exposed to stress effects. These reactions seem to be general strategies to improve stress tolerance (28). However, very little information is available on the role of the ascorbate-GSH cycle in plant defense reactions after pathogen attack (11,31).

Glutathione S-transferase (GST, EC 2.5.1.18) isoenzymes have a well-defined role in plant detoxification reactions. They are capable of catalyzing the binding of various xenobiotics and their electrophilic metabolites with GSH to produce less-toxic conjugates (19,23). Various abiotic stress effects are powerful inducers of GST activity in plants (up to 2,800% of the control) (5,12). GST isoenzymes are also antioxidative enzymes. They catalyze the breakdown of fatty acid hydroperoxides and contribute to protection against oxidative membrane damage and necrotic disease symptoms (2,20). The increased transcription of genes-encoding GST isoenzymes has been found in different plants after fungal infections. Winter wheat plants infected by the nonpathogen of *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal showed local, induced resistance against a second infection with the pathogen *E. graminis* f. sp. *tritici*. One of the genes activated simultaneously with the onset of resistance encoded a GST isoenzyme (7). Also, different GST isoenzymes were induced in wheat by xenobiotics and by powdery mildew infection (23). Recently, the defense gene *prp 1-1* has been shown to encode a GST isoenzyme in potato (13).

To gain more information about the possible role of the ascorbate-GSH antioxidative cycle in plant defense reactions against pathogens, we have investigated the effects of artificial powdery mildew infections on the rate of lipid peroxidation (malondialdehyde content), on ascorbate and GSH levels, and on AP, DHAR, and GR activities in barley. The glutathione-dependent GST activity also was measured. Barley (*Hordeum vulgare* L.) cultivars showing different degrees of susceptibility were used to investigate various host-pathogen interactions. Only minor changes in soluble superoxide dismutase (SOD, EC 1.15.1.1.) activity were found in mildewed leaves of these barley plants (1). In additional

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experiments, infections of one barley cultivar with two powdery mildew races (compatible or incompatible interactions) also were studied.

MATERIALS AND METHODS

Plants and pathogens. Seeds of three barley cultivars (Amsel, Emir, and GK-Omega) were planted in soil and grown under normal greenhouse conditions (temperature: 18 to 23°C; supplemental light: 160 $\mu E\ m^{-2}\ s^{-1}$ for 8 h/day; relative humidity: 75 to 80%). Leaves of 7-day-old seedlings were artificially inoculated by an isolate of *E. graminis* f. sp. *hordei*. This Hungarian isolate of the fungus was obtained from an infected barley field in Hungary and maintained on barley seedlings in the greenhouse. Infections were initiated by gently shaking conidia from the leaves of donor plants onto leaves of the barley cultivars examined. Based on visual symptoms, Emir was classified as highly susceptible, GK-Omega as moderately susceptible, and Amsel as resistant against the Hungarian powdery mildew isolate.

In separate experiments, one barley cultivar (Amsel) was infected with two mildew races. The incompatible reaction caused by the Hungarian isolate was compared with the compatible reaction brought about by barley powdery mildew race C17 Am (obtained from M. Niemann, Institute for Plant Pathology, University of Göttingen, Göttingen, Germany).

The primary (oldest) leaves of seedlings were used for biochemical analyses after different time periods (1 to 6 days) following infection. Approximately 8 to 10 plants were used for each analysis.

Enzyme activity assays. For enzyme assays, cell-free homogenates were prepared at 0 to 4°C. Infected and control leaves (0.5 g) were frozen in liquid nitrogen, pulverized with a mortar and pestle, and suspended in 3 ml of cold 0.2 M TRIS/HCl buffer (pH 7.8) containing 3% soluble polyvinylpyrrolidone and 0.1 mM Na_2-EDTA. The homogenate was strained through muslin and centrifuged at 8,000 × *g* for 20 min. The supernatants were used as the enzyme source.

Spectrophotometric methods were used to determine the various enzyme activities. AP activity was determined according to the method of Nakano and Asada (26). The oxidation of ascorbic acid was determined at 290 nm. The concentrations of H_2O_2 and ascorbic acid were 1 and 0.25 mM in the reaction mixture, respectively. DHAR and GR activities were measured by determining the reduction of dehydroascorbate (at 265 nm) or oxidized glutathione (at 340 nm), respectively (17). GST activity was determined by measuring the formation of the conjugate reaction product at 340 nm using 1-chloro-2,4-dinitrobenzene as a substrate (18). One-electron (SOD inhibitable) and two-electron (dicoumarol inhibitable DT-diaphorase, EC 1.6.99.2) quinone reductase (QR) activities were measured using menadione as a substrate, according to previously published methods (references 3 and 21, respectively).

Other assays. Lipid peroxidation was detected by measuring malondialdehyde (MDA) levels with the 2-thiobarbituric acid reagent (29). Acid-soluble nonprotein thiol levels were determined spectrophotometrically with 5,5′-dithiobis-2-nitrobenzoic acid, according to De Kok and Graham (6). Ascorbic acid was measured using ascorbate oxidase (38). The quantification of fungal material in the leaves was based on glucosamine (chitosan) analysis (32).

Statistics. At least three independent parallel experiments were carried out for each treatment. The significant differences between mean values were evaluated by Student's *t* test. Differences were considered to be significant at $P = 0.05$.

RESULTS

Initial symptoms appeared on the leaves of barley cultivars Emir and GK-Omega on the third day after inoculation with the Hungarian isolate of *E. graminis* f. sp. *hordei*. Sporulation of the fungus was much more rapid on Emir than on GK-Omega. On the leaves of cultivar Amsel, hypersensitive necrotic spots appeared 4 days after inoculation. To compare fungal growth rates in the three barley cultivars quantitatively, the amount of mycelium produced in the leaves was determined by assaying for glucoseamine content (Fig. 2). The results of these experiments supported earlier visual classifications. The highest fungal content was found in infected leaves of highly susceptible Emir from the fourth day after infection. The fungus was less virulent on GK-Omega, whereas only a very small amount of mycelium was detected in resistant Amsel.

To characterize lipid peroxidation processes (which are the consequences of oxidative stress) in barley leaves, MDA levels were

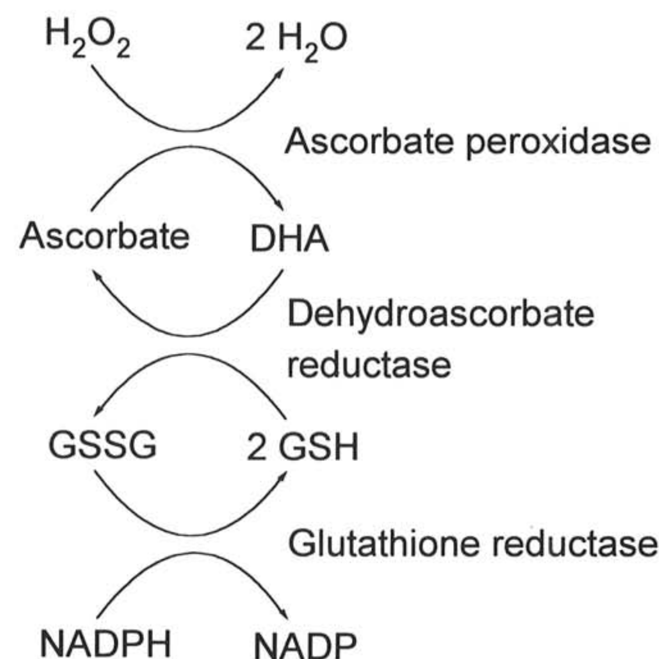

Fig. 1. Reduction of hydrogen peroxide by the chloroplastic ascorbate-glutathione cycle.

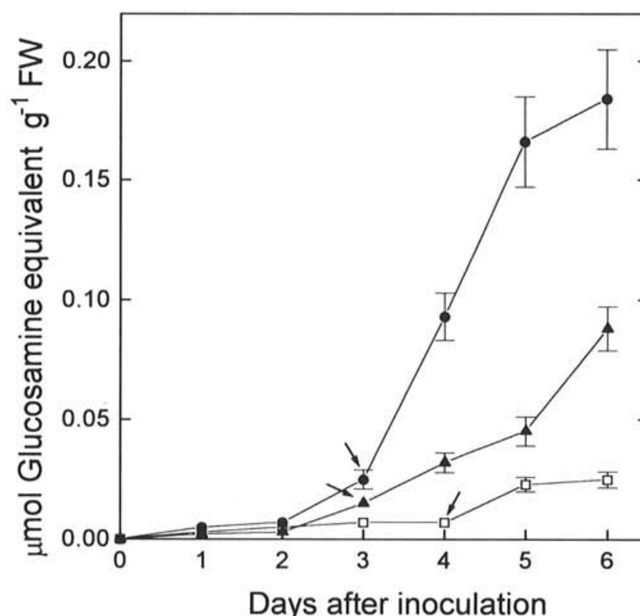

Fig. 2. Fungal mycelium (measured as glucosamine content) in leaves of three barley cultivars inoculated with *Erysiphe graminis* f. sp. *hordei*. Means of three replicate experiments ± SD are shown. Arrows indicate the first appearance of visible symptoms. Cv. Amsel (resistant) = —□—; cv. GK-Omega (moderately susceptible) = —▲—; cv. Emir (highly susceptible) = —●—.

measured after fungal infection. Markedly increased MDA levels were found in infected leaves of the resistant cultivar Amsel (Fig. 3). The MDA level started to rise at 4 days after infection, and after 6 days, it reached 185% of the uninfected control value. MDA levels did not change significantly in mildewed leaves of the susceptible cultivars Emir and GK-Omega (Fig. 3).

The ascorbic acid content decreased in all cultivars after infection. A substantial, gradual decrease in ascorbic acid level (down to 38% of uninfected control) was observed in highly susceptible Emir (Fig. 4). A less-pronounced decrease was found in GK-Omega. In contrast to the susceptible cultivars, the slight decrease in the ascorbic acid level stopped in the infected resistant Amsel leaves at 4 days after infection, and then the ascorbic acid concentration returned to the control level.

Powdery mildew infection substantially elevated AP activity in leaves of the susceptible cultivars Emir and GK-Omega (Table 1). Less noticeable, but still significant, increases in AP activity were found in the resistant cultivar Amsel. In contrast to AP activity, declining DHAR activity was observed in inoculated leaves. We found marked decreases in DHAR activity in susceptible Emir and GK-Omega but only small reductions in resistant Amsel (Table 1).

Barley leaves contain significant amounts of τ-glutamylcysteinylserine in addition to GSH (16). Since the GSH homologue also can participate in the ascorbate-GSH cycle (16), we determined the total acid-soluble nonprotein thiol levels as an estimate of τ-glutamylcysteinylserine and GSH content in mildewed barley leaves. Contrary to the ascorbic acid results, the nonprotein thiol content increased markedly 3 and 4 days after infection in leaves of the moderately susceptible cultivar GK-Omega, reaching 156% of untreated controls. After this period, the thiol content declined (Fig. 5). In the resistant cultivar Amsel, the thiol content began to increase 2 days after infection, and marked increases were found 5 and 6 days after inoculation. No significant change was found in the highly susceptible cultivar Emir (Fig. 5).

GR activity was not influenced significantly by infection with *E. graminis* f. sp. *hordei* in any of the three cultivars (data not shown). However, significant alterations were found in the NADPH-consuming activity of barley leaf cell-free extracts (measurement of the NADPH-consuming activity is a necessary control determination in each GR assay). NADPH-consuming activity increased only slightly in resistant Amsel and in moderately susceptible GK-Omega. However, in highly susceptible Emir, this activity began to increase dramatically 5 days after inoculation, and after 6 days, it was about five times higher compared to the untreated control (Fig. 6).

One explanation for the increase in NADPH consumption could be the reduction of quinones to phenols by NADPH, catalyzed by QR (21). To test this hypothesis, we measured the NADPH-dependent DT-diaphorase activity in diseased barley leaves. The dicoumarol-inhibitable DT-diaphorase activity (two-electron QR) could not be detected in extracts from diseased and healthy leaves of any of the cultivars studied (data not shown). However, we observed the one-electron reduction of the quinone menadione in bar-

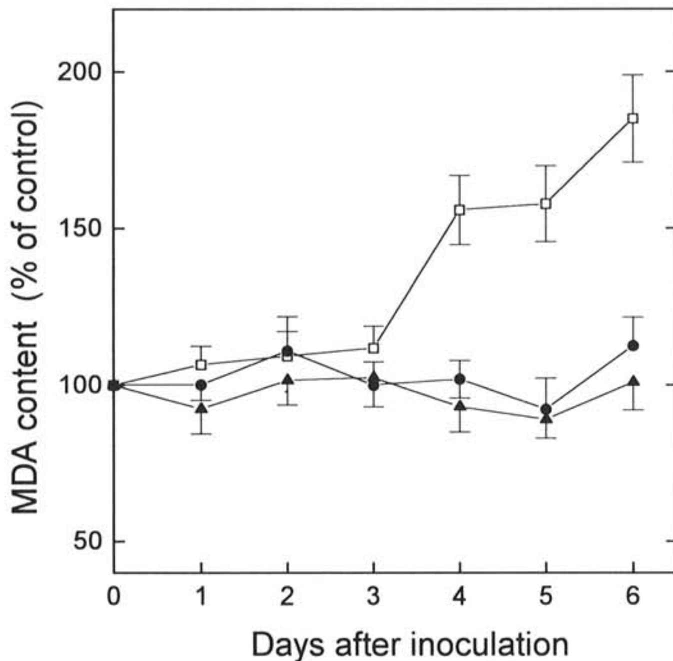

Fig. 3. Malondialdehyde (MDA) levels in leaves of three barley cultivars inoculated with *Erysiphe graminis* f. sp. *hordei*. The MDA content of control plants was 14.9 ± 1.1 (cv. Amsel), 16.7 ± 1.4 (cv. GK-Omega), and 18.1 ± 1.6 (cv. Emir) nmol MDA g^{-1} fresh weight ($n = 5$). Means of three replicate experiments ± SD are shown. Amsel (resistant) = —□—; GK-Omega (moderately susceptible) = —▲—; Emir (highly susceptible) = —●—.

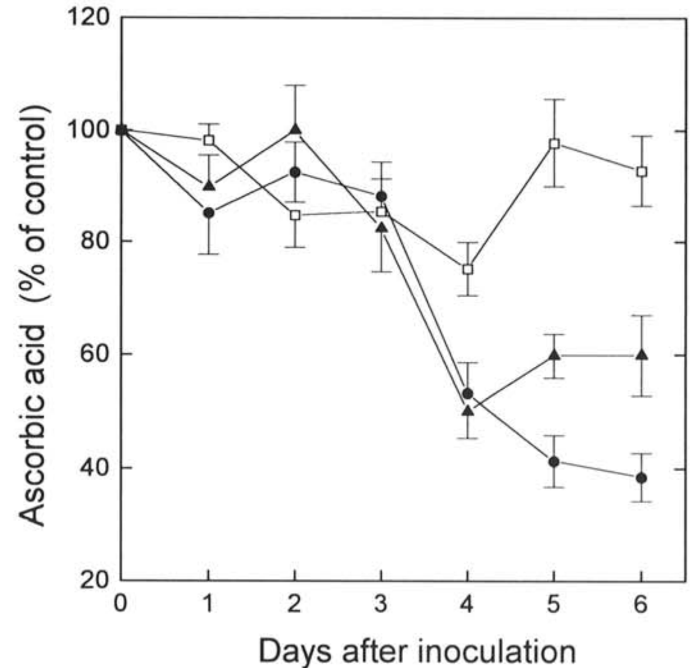

Fig. 4. Ascorbic acid levels in leaves of three barley cultivars after inoculation with *Erysiphe graminis* f. sp. *hordei*. The ascorbic acid content of control plants was 2.08 ± 0.33 (cv. Amsel), 2.19 ± 0.43 (cv. GK-Omega), and 2.05 ± 0.39 (Emir) μmol ascorbic acid g^{-1} fresh weight ($n = 5$). Means of three replicate experiments ± SD are shown. Amsel (resistant) = —□—; GK-Omega (moderately susceptible) = —▲—; Emir (highly susceptible) = —●—.

TABLE 1. Changes in ascorbate peroxidase (AP) and dehydroascorbate reductase (DHAR) activities[z] in leaves of three barley cultivars infected with *Erysiphe graminis* f. sp. *hordei*

Enzyme	Days after infection	Activity (% of uninfected control)		
		Amsel	GK-Omega	Emir
AP	4	121 a	191 b	183 b
	5	160 a	242 b	275 b
	6	136 a	227 b	307 c
DAHR	4	76 a	63 a	37 b
	5	78 a	47 b	31 c
	6	74 a	47 b	57 b

[z] Data presented are the means of three replicate experiments ± SD 6 to 11%. In each row, values followed by the same letter are not significantly different at $P = 0.05$ (all activities differed significantly from uninfected controls). AP activity in untreated control leaves was (mean ± SD) 3.5 ± 0.3, 3.1 ± 0.2, and 2.9 ± 0.2 μmol ascorbate g^{-1} fresh weight min^{-1} in cultivars Amsel (resistant), GK-Omega (moderately susceptible), and Emir (highly susceptible), respectively. DHAR activity in untreated control leaves was 11.3 ± 1.4, 12.7 ± 1.0, and 11.4 ± 0.9 μmol ascorbate g^{-1} fresh weight min^{-1} in Amsel, GK-Omega, and Emir, respectively. Control values remained unchanged over the time course of the experiment.

ley extracts (Table 2). This SOD-inhibitable activity was presumably due to the NADPH-cytochrome P-450 reductase enzyme (3). The one-electron QR activity significantly decreased in the inoculated leaves of the susceptible plants, mostly in Emir (Table 2), showing that the increased NADPH consumption was not due to induced QR activity.

GST activity was markedly induced after *E. graminis* f. sp. *hordei* inoculation in each barley cultivar, but the rate and timing of the induction were different among the cultivars. The most substantial rate of induction was found in the highly susceptible cultivar Emir (Fig. 7). The enzyme activity increased gradually up to the end of the experimental period, and 6 days after infection it reached 360% of the uninfected control. A high rate of induction also was observed in the moderately susceptible cultivar GK-Omega. In the resistant cultivar Amsel, GST activity increased up to 3 days after infection (up to 157% of the control) but then declined.

In additional experiments, seedlings of one cultivar (Amsel) were inoculated with the Hungarian isolate (incompatible reaction) and with barley powdery mildew race C17 Am (compatible reaction). The changes in the level of three antioxidants (ascorbic acid, AP, and GST) were compared in two host-pathogen interactions with the same cultivar. The results obtained with these two races confirmed our earlier findings: the compatible interaction led to a dramatic decrease in ascorbic acid level and strongly induced AP and GST activities. In the incompatible host-fungus interaction, small or no alterations were observed in these antioxidant levels (Table 3).

DISCUSSION

The significantly increasing MDA content of mildewed leaves in the incompatible host-fungus interaction showed that lipid peroxidation occurred concomitantly with the appearance of necrotic symptoms (Fig. 3). However, the level of the antioxidant ascorbic acid decreased to a much lesser extent in these resistant leaves than in compatible interactions in which increased MDA levels could not be detected. The marked decline of ascorbic acid in compatible interactions may be explained by the strong induction of AP activity in the infected plants. Since the reducing equivalents for the regeneration of ascorbic acid are channeled through the ascorbate-GSH cycle from NADPH, the elevated AP activity might contribute to the dramatic increase in NADPH-consuming activity observed in susceptible Emir leaves. However, if the decreased DHAR and unchanged GR activities observed in these leaves are rate-limiting in the function of the cycle, then decreasing DHAR activity also may contribute to the decline of ascorbic acid levels. Another possible explanation of NADPH consumption could be the induction of a QR enzyme. However, our experiments did not support this hypothesis. The decreased one-electron QR activity observed in the susceptible cultivar Emir may contribute to the decline of superoxide production (3) and, thereby, to the suppression of necrotic processes in the susceptible host.

An early report demonstrated the antioxidative and protective effect of ascorbic acid and GSH against the development of necrotic symptoms in virus-infected plants (9). Recently, the intensive study of oxidative stress in plant-pathogen interactions has led to a deeper insight into the mechanism of antioxidative plant defense

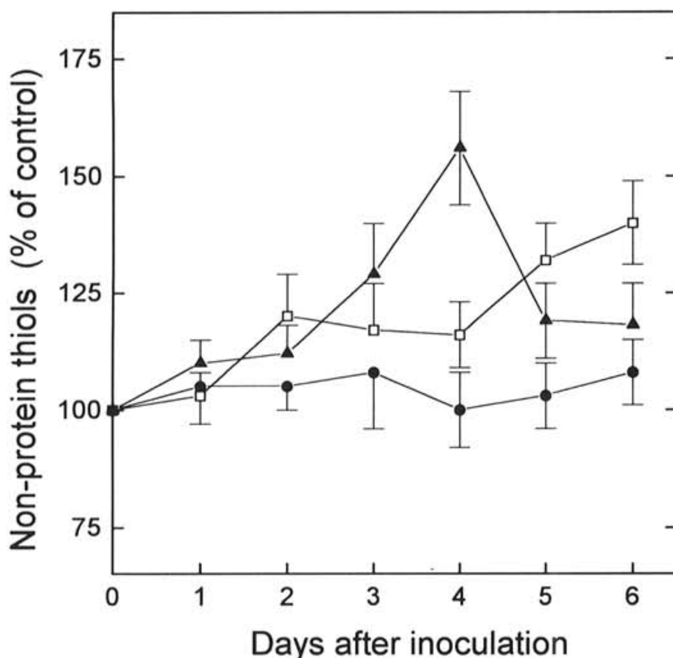

Fig. 5. Nonprotein thiol levels in leaves of three barley cultivars after inoculation with *Erysiphe graminis* f. sp. *hordei*. The thiol content of control plants was 0.39 ± 0.03 (cv. Amsel), 0.41 ± 0.04 (cv. GK-Omega), and 0.37 ± 0.02 (cv. Emir) µmol thiol g^{-1} fresh weight (n = 6). Means of three replicate experiments ± SD are shown. Amsel (resistant) = —□—; GK-Omega (moderately susceptible) = —▲—; Emir (highly susceptible) = —●—.

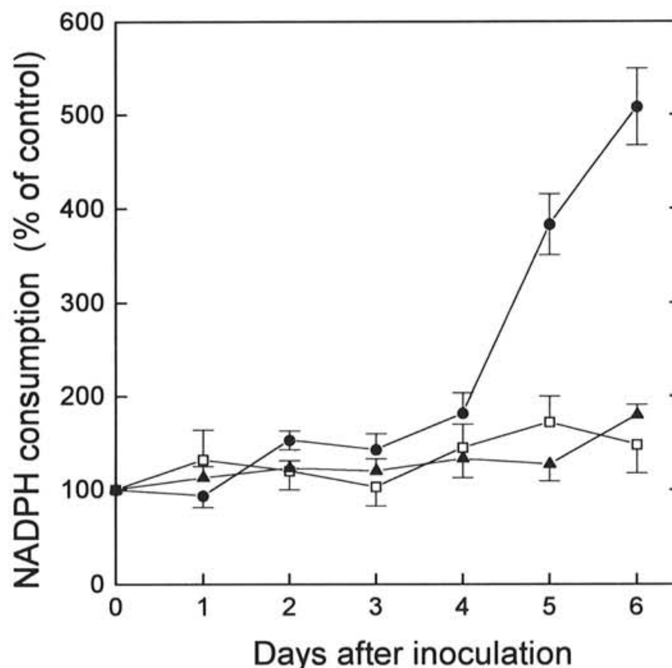

Fig. 6. Changes in the NADPH-consuming activity in barley leaves after inoculation with *Erysiphe graminis* f. sp. *hordei*. The NADPH-consuming activity in the control plants was 0.096 ± 0.018 (cv. Amsel), 0.094 ± 0.028 (cv. GK-Omega), and 0.090 ± 0.021 (cv. Emir) µmol NADPH g^{-1} fresh weight min^{-1} (n = 5). Means of three replicate experiments ± SD are shown. Amsel (resistant) = —□—; GK-Omega (moderately susceptible) = —▲—; Emir (highly susceptible) = —●—.

TABLE 2. Soluble superoxide dismutase-inhibitable one-electron quinone reductase activity in leaves of three barley cultivars infected with *Erysiphe graminis* f. sp. *hordei*

Days after infection	Activity[z] (% of uninfected control)		
	Amsel	GK-Omega	Emir
4	95 a	102 a	25 b
5	120 a	78 b	21 c
6	117 a	35 b	35 b

[z] Data presented are the means of three replicate experiments ± SD 7 to 13%. In each row, values followed by the same letter are not significantly different at P = 0.05. Activity in untreated control leaves was (mean ± SD) 11.4 ± 1.3, 11.5 ± 0.9, and 11.8 ± 1.0 nmol reduced cytochrome C g^{-1} fresh weight min^{-1} in cultivars Amsel (resistant), GK-Omega (moderately susceptible), and Emir (highly susceptible), respectively. Control values remained unchanged over the time course of the experiment.

reactions. Exogenous GSH stimulated the transcription of genes encoding enzymes of phytoalexin and lignin biosynthesis in bean suspension cultures (37). Decreased glutathione and increased ascorbate levels were found in *Avena sativa* after infection with virulent *Drechslera* species (11). Interestingly, opposite trends were found in our experiments. Increasing nonprotein thiol and decreasing ascorbic acid levels were determined in susceptible barley plants infected with *E. graminis* f. sp. *hordei*, which is a biotrophic fungus, in contrast to the necrotrophic *Drechslera* (*Helminthosporium*). Probably, in the case of biotrophic parasitism, when infected tissues remain alive for a long time, stress reactions that are activated as a result of the infection are different from those activated after infection with a necrotizing pathogen. The dramatic decrease in ascorbic acid and the concomitant apparent stability of thiol content in the heavily infected susceptible cultivar Emir show that ascorbic acid may play a dominant role in antioxidative reactions against necrotic processes associated with powdery mildew infection.

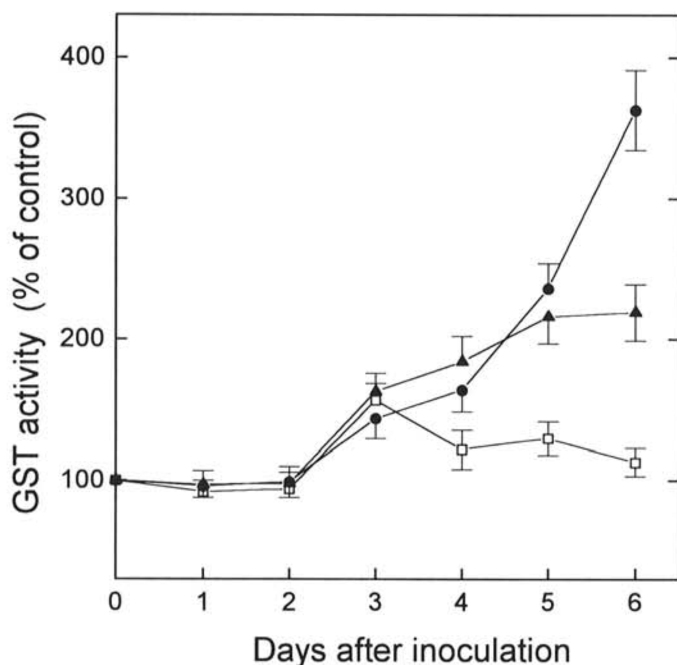

Fig. 7. Induction of glutathione S-transferase (GST) activity in leaves of three barley cultivars inoculated with *Erysiphe graminis* f. sp. *hordei*. The GST activity in control plants was 0.66 ± 0.08 (cv. Amsel), 0.54 ± 0.10 (cv. GK-Omega), and 0.56 ± 0.08 (cv. Emir) μmol conjugate g^{-1} fresh weight min^{-1} (n = 5). Means of three replicate experiments ± SD are shown. Amsel (resistant) = —□—; GK-Omega (moderately susceptible) = —▲—; Emir (highly susceptible) = —●—.

TABLE 3. Ascorbic acid level and ascorbate peroxidase (AP) and glutathione S-transferase (GST) activities in leaves of the barley cultivar Amsel infected with *Erysiphe graminis* f. sp. *hordei*, Hungarian isolate or race C17 Am

	Percent of uninfected control[z]					
	Hungarian isolate (incompatible interaction)			C17 Am (compatible interaction)		
Enzyme	4 DAI	5 DAI	6 DAI	4 DAI	5 DAI	6 DAI
Ascorbic acid	76 ± 7	98 ± 8	93 ± 8	52 ± 6	27 ± 4	22 ± 5
AP	121 ± 9	159 ± 13	136 ± 10	289 ± 18	588 ± 44	428 ± 41
GST	128 ± 11	125 ± 14	117 ± 11	235 ± 17	384 ± 31	339 ± 28

[z] Data presented are the means of three replicate experiments ± SD. Antioxidant levels and activities in untreated control leaves were 1.94 ± 0.13 μmol ascorbic acid g^{-1} fresh weight, 3.2 ± 0.2 μmol ascorbate g^{-1} fresh weight min^{-1}, and 0.61 ± 0.05 μmol conjugate g^{-1} fresh weight min^{-1} for ascorbic acid, AP, and GST, respectively (n = 6). Control values remained unchanged over the time course of the experiment. DAI = days after inoculation.

In winter wheat, an approximate 20-fold increase in the level of a mRNA encoding a GST isozyme was detected after infection by a nonpathogenic powdery mildew, *E. graminis* f. sp. *hordei* (7,23). We found heavily increased GST activity in the susceptible barley cultivars and, to a lesser extent, also in the resistant cultivar. It is supposed that GST, by its GSH peroxidase activity, participates in the detoxification of fatty acid hydroperoxides produced during lipid peroxidation processes (2,20). Since fungi are known to contain GST enzymes (4,27), both the host and pathogen may contribute to increases in GST activity. Because of the obstacles in preparing in vitro powdery mildew cultures, we have estimated the possible fungal contribution from fungal growth data. Figure 2 shows that the ratio between the fungal mycelium contents in Emir and Amsel leaves was about 7:1 6 days after infection. At the same time, the increase in GST activity in diseased resistant Amsel leaves was 13%. In the highly improbable case that all the additional activity originated from the fungus, GST activity in the susceptible Emir leaves would be elevated by 91%. However, the GST activity was 3.6 times higher in the infected leaves than in the control leaves. Obviously, the fungal contribution was not significant in the induction of these enzymes. Estimations based on AP and NADPH-consuming activities gave similar results (data not shown). The fungal contribution to the activity of some antioxidative enzymes was less than 5% in oat leaves infected by *Drechslera* spp. (11). The increased lipoxygenase activity in powdery mildew-infected tobacco leaves also originated in the host plant (22).

Earlier studies showed that symptoms of infection by *E. graminis* f. sp. *hordei* were inhibited in barley plants by an exogenous superoxide-producing system (14). It is supposed that the pathogen also is inhibited or killed in planta if an oxygen radical-producing system is present (15). Our results provide evidence that *E. graminis* f. sp. *hordei* infection leads to significant changes in the antioxidant metabolism of barley. In the compatible interaction, the fungus grows rapidly in the leaf tissue affecting numerous cells. Thus, the stronger induction of antioxidative enzymes may be explained by the fact that the fungus elicits a stress reaction from more cells than in an incompatible reaction. However, no increase of lipid peroxidation was observed in the compatible interaction (with substantially decreasing ascorbic acid levels), and in the incompatible interaction, in which fewer cells were stressed, the rate of lipid peroxidation was intensively elevated. This suggests that the induced antioxidative processes are probably not only stress reactions, but that they also can contribute to the suppression of lipid peroxidation and necrotic symptom expression. In compatible host-parasite interactions, several reactions seem to be important: (i) the antioxidative ascorbate-GSH cycle, indicated by the substantial decrease in ascorbate levels; (ii) GST enzymes, which may diminish the damaging effects of lipid peroxidation; and (iii) the decrease in one-electron QR activity.

Further investigations are needed to determine whether our results support the induced susceptibility theory of host-biotrophic pathogen interactions. From our experiments, we can only conclude that in compatible barley-powdery mildew interactions the lipid peroxidation is insignificant and antioxidant reactions are induced that inhibit tissue necrotization. There exists a correlation between susceptibility and suppression of necrosis by antioxidative reactions in barley; however, correlation does not necessarily imply causation.

LITERATURE CITED

1. Ádám, A., Érsek, T., and Király, Z. 1989. Quantification of the activity of superoxide dismutase by densitometric analysis of acrylamide gels: Application to mildewed barley. Acta Phytopathol. Entomol. Hung. 24:259-265.
2. Bartling, D., Radzio, R., Steiner, U., and Weiler, E. W. 1993. A glutathione S-transferase with glutathione-peroxidase activity from *Arabidopsis thaliana*: Molecular cloning and functional characterization. Eur. J. Biochem. 216: 579-586.

3. Chesis, P. L., Levin, D. E., Smith, M. T., Ernster, L., and Ames, B. N. 1984. Mutagenicity of quinones: Pathways of metabolic activation and detoxification. Proc. Natl. Acad. Sci. USA 81:1696-1700.
4. Cohen, E., Gamliel, A., and Katan, J. 1986. Glutathione and glutathione S-transferase in fungi: Effect of pentachloronitrobenzene and 1-chloro-2,4-dinitrobenzene; purification and characterization of the transferase from *Fusarium*. Pestic. Biochem. Physiol. 26:1-9.
5. Dean, J. V., Gronwald, J. W., and Eberlein, C. V. 1990. Induction of glutathione S-transferase isozymes in sorghum by herbicide antidotes. Plant Physiol. 92:467-473.
6. De Kok, L. J., and Graham, M. 1989. Levels of pigments, soluble proteins, amino acids and sulfhydryl compounds in foliar tissue of *Arabidopsis thaliana* during dark-induced and natural senescence. Plant Physiol. Biochem. 27:203-209.
7. Dudler, R., Hertig, C., Rebmann, G., Bull, J., and Mauch, F. 1991. A pathogen-induced wheat gene encodes a protein homologous to glutathione-S-transferases. Mol. Plant-Microbe Interact. 4:14-18.
8. Edwards, E. A., Enard, C., Creissen, G. P., and Mullineaux, P. M. 1994. Synthesis and properties of glutathione reductase in stressed peas. Planta 192:137-143.
9. Farkas, G. L., Király, Z., and Solymosi, F. 1960. Role of oxidative metabolism in the localization of plant viruses. Virology 12:408-421.
10. Foyer, C. H., Descourvieres, P., and Kunert, K. J. 1994. Protection against oxygen radicals: An important defense mechanism studied in transgenic plants. Plant Cell Environ. 17:507-523.
11. Gönner, M. v., and Schlösser, E. 1993. Oxidative stress in interactions between *Avena sativa* L. and *Drechslera* spp. Physiol. Mol. Plant Pathol. 42:221-234.
12. Gullner, G., Kömives, T., and Király, L. 1991. Enhanced inducibility of antioxidant systems in a *Nicotiana tabacum* L. biotype results in acifluorfen resistance. Z. Naturforsch. Teil C Biochem. Biophys. Biol. Virol. 46:875-881.
13. Hahn, K., and Strittmatter, G. 1994. Pathogen-defense gene *prp 1-1* from potato encodes an auxin-responsive glutathione S-transferase. Eur. J. Biochem. 226:619-626.
14. Király, Z., El-Zahaby, H., Galal, A., Abdou, S., Ádám, A., Barna, B., and Klement, Z. 1993. Effect of oxy free radicals on plant pathogenic bacteria and fungi and on some plant diseases. Pages 9-19 in: Oxygen Free Radicals and Scavengers in the Natural Sciences. Gy. Mózsik, I. Emerit, J. Fehér, B. Matkovics, and Á. Vincze, eds. Akadémiai Kiadó, Budapest.
15. Király, Z., Érsek, T., Barna, B., Ádám, A., and Gullner, G. 1991. Pathophysiological aspects of plant disease resistance. Acta Phytopathol. Entomol. Hung. 26:233-250.
16. Klapheck, S., Chrost, B., Starke, J., and Zimmermann, H. 1992. τ-Glutamylcysteinylserine—A new homologue of glutathione in plants of the family *Poaceae*. Bot. Acta 105:174-179.
17. Klapheck, S., Zimmer, I., and Cosse, H. 1990. Scavenging of hydrogen peroxide in the endosperm of *Ricinus communis* by ascorbate peroxidase. Plant Cell Physiol. 31:1005-1013.
18. Kömives, A. V., Kömives, T., and Dutka, F. 1985. Effects of thiocarbamate herbicides on the activity of glutathione S-transferase in maize. Cereal Res. Commun. 13:253-257.
19. Lamoureux, G. L., and Rusness, D. G. 1989. The role of glutathione and glutathione S-transferases in pesticide metabolism, selectivity, and mode of action in plants and insects. Pages 154-196 in: Coenzymes and Cofactors. Vol. 3., part B. D. Dolphin, R. Poulson, and O. Avramovic, eds. John Wiley and Sons, New York.
20. Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. 1994. H_2O_2 from oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79:583-593.
21. Lind, C., Cadenas, E., Hochstein, P., and Ernster, L. 1990. DT-diaphorase: Purification, properties, and function. Pages 287-301 in: Methods in Enzymology. Vol. 186. S. P. Colowick and N. O. Kaplan, eds. Academic Press, New York.
22. Lupu, R., Grossman, S., and Cohen, Y. 1980. The involvement of lipoxygenase and antioxidants in pathogenesis of powdery mildew on tobacco plants. Physiol. Plant Pathol. 16:241-248.
23. Mauch, F., and Dudler, R. 1993. Differential induction of distinct glutathione-S-transferases of wheat by xenobiotics and by pathogen attack. Plant Physiol. 102:1193-1201.
24. Mehdy, M. C. 1994. Active oxygen species in plant defense against pathogens. Plant Physiol. 105:467-472.
25. Nakagawara, S., and Sagisaka, S. 1984. Increases in enzyme activities related to ascorbate metabolism during cold acclimation in poplar twigs. Plant Cell Physiol. 25:899-906.
26. Nakano, Y., and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22:867-880.
27. Oros, Gy., and Kömives, T. 1991. Effects of phenylamide pesticides on the GSH-conjugation system of *Phytophthora* spp. fungi. Z. Naturforsch. Teil C Biochem. Biophys. Biol. Virol. 46:866-874.
28. Polle, A., and Rennenberg, H. 1993. Significance of antioxidants in plant adaptation to environmental stress. Pages 263-273 in: Plant Adaptation to Environmental Stress. L. Fowden, T. Mansfield, and J. Stoddart, eds. Chapman and Hall, London.
29. Popham, P. L., and Novacky, A. 1991. Use of dimethyl sulfoxide to detect hydroxyl radical during bacteria-induced hypersensitive reaction. Plant Physiol. 96:1157-1160.
30. Ranieri, A., Lencioni, L., Schenone, G., and Soldatini, G. F. 1993. Glutathione-ascorbic acid cycle in pumpkin plants grown under polluted air in open-top chambers. J. Plant Physiol. 142:286-290.
31. Rennenberg, H., and Brunold, C. 1994. Significance of glutathione metabolism in plants under stress. Pages 142-156 in: Progress in Botany. Vol. 55. H.-D. Behnke, U. Lüttge, K. Esser, J. W. Kadereit, and M. Runge, eds. Springer-Verlag, Berlin.
32. Ride, J. P., and Drysdale, R. B. 1972. A rapid method for the chemical estimation of filamentous fungi in plant tissue. Physiol. Plant Pathol. 2: 7-15.
33. Schmidt, A., and Kunert, K. J. 1986. Lipid peroxidation in higher plants. Plant Physiol. 82:700-702.
34. Smith, I. K., Polle, A., and Rennenberg, H. 1990. Glutathione. Pages 201-215 in: Stress Responses in Plants: Adaptation and Acclimation Mechanisms. R. G. Alscher and J. R. Cumming, eds. Wiley-Liss, Inc., New York.
35. Sutherland, M. W. 1991. The generation of oxygen radicals during host plant responses to infection. Physiol. Mol. Plant Pathol. 39:79-93.
36. Tzeng, D. D., and DeVay, J. E. 1993. Role of oxygen radicals in plant disease development. Pages 1-34 in: Advances in Plant Pathology. Vol. 10. J. H. Andrews and I. C. Tommerup, eds. Academic Press, London.
37. Wingate, V. P. M., Lawton, M. A., and Lamb, C. J. 1988. Glutathione causes a massive and selective induction of plant defense genes. Plant Physiol. 87:206-210.
38. Wise, R. R., and Naylor, A. W. 1987. Chilling enhanced photooxidation. Evidence for the role of singlet oxygen and superoxide in the breakdown of pigments and endogenous antioxidants. Plant Physiol. 83:278-282.