

## Sugar Composition and Acid Invertase Activity in Spring Barley Plants in Relation to Adult-Plant Resistance to Powdery Mildew

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### ABSTRACT

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Ethanol-soluble sugars and acid invertase in healthy and powdery mildew-infected leaves of the susceptible cultivar Peruvian and the adult-plant-resistant cultivar Asse were quantitatively analyzed. A slight increase in glucose, fructose, and sucrose in infected first leaves of barley plants at the first leaf stage appeared at 2-4 days after infection in both cultivars. During sporulation, glucose and fructose increased more in Peruvian than in Asse. Sucrose, in contrast, accumulated in infected leaves of Asse, showing a large increase at 9 days after inoculation. The amounts of fructose and glucose in infected third leaves at the four-leaf stage increased more in Peruvian than in Asse. However, levels of sucrose in infected leaves were greater in Asse than in Peruvian. Increased levels of glucose and

fructose in plants of the two cultivars were closely correlated with increases in acid invertase activity. In noninfected fourth leaves of plants with the lower three leaves infected, the amounts of fructose and glucose declined and the amount of sucrose increased in both cultivars. However, the decline was more marked in Peruvian than Asse, whereas sucrose increased more in Asse. The changes in sugar content and activity of acid invertase in infected first and third leaves of the two cultivars were closely associated with infection intensity of powdery mildew. These results suggest that carbohydrate metabolism is less altered in powdery mildew-infected plants of adult-plant-resistant barley cultivars than in susceptible cultivars.

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Substantial changes in carbohydrate content of plants infected by biotrophic fungi reflect the alteration in various metabolic processes favorable or unfavorable for fungal development. Each

of the principal metabolic processes like photosynthesis, respiration, and translocation can be affected by the pathogen, producing profound imbalance, sometimes even in noninfected parts of the plant (14).

Allen (1) reported an accumulation of soluble sugars in powdery mildew-infected wheat. The amounts of the carbohydrates in infected plants vary with the stage of fungal development and infection intensity (5,8,13). A close correlation between low sucrose content in infected plant tissues and a high activity of acid invertase has been observed (3,15,17).

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In previous investigations, qualitative and quantitative differences in resistance to powdery mildew between spring barley cultivars during plant development could be easily detected under controlled conditions on the basis of race specificity, infection type or rate (9), and infection process (10). Carbohydrate metabolism altered during plant development, possibly with some relation to the appearance of adult plant resistance in the barley cultivars tested (11). We also suggested that gene action may be changed during development in barley cultivars showing different levels of resistance to powdery mildew (12). The question remained whether the amounts of soluble sugars and invertase activity in these host-parasite systems of different compatibility are altered.

This research was initiated to evaluate the changes in soluble sugars and acid invertase activity in powdery mildew infected plants of susceptible and adult-plant-resistant barley cultivars.

## MATERIALS AND METHODS

**Plant and inoculum.** The spring barley (*Hordeum vulgare* L.) cultivars used were Peruvian, which is susceptible at all plant growth stages, and Asse, which is susceptible at early growth stages but resistant at late growth stages. To avoid any contamination by powdery mildew, the plants were grown in cases made of glass and muslin in a greenhouse as described earlier (11). Plants at the required stages of development were inoculated by uniformly

spraying a conidial suspension of *Erysiphe graminis* f. sp. *hordei* Marchal (race C<sub>17</sub> Amsel) in FC 43 (Fluorinert Electronic Liquid, Commercial Chemicals Division/3M, St. Paul, MN) on the abaxial surface of the leaves. The same inoculum density was sprayed on the seedling leaves of both cultivars. As a result, the leaf areas infected were about 40% for Peruvian and about 20% for Asse. The infection types for Peruvian were 4 and 4 (highly susceptible), but for Asse 4 and 2-3 (2 = moderately resistant, and 3 = susceptible) at the first and fourth leaf stages, respectively (9). The plants showing different numbers of colonies after inoculation (Fig. 1) were selected for determining the effect of infection intensities on the metabolism of barley plants at the first and fourth leaf stages. The number of colonies on the basis of leaf area was measured 4-6 days after inoculation because of difficulties in counting colonies thereafter.

**Analysis of sugars.** One gram (fresh weight) of leaf tissue at the required stage of development was harvested at 1100 hours, cut into 1-cm segments and boiled in 20 ml of 80% (v/v) ethanol (three changes). Prior to sampling, the conidia and mycelium produced were carefully removed by brushing the infected leaves. The pigments were removed from the ethanol extracts by shaking in 80 ml of 80% ethanol:petroleum benzene (5:3, v/v). Total sugars were determined in aliquots of the ethanol-water fractions by the method of Dubois et al (6). The remaining ethanol-soluble fractions were stored at -15 C until analyzed for individual sugars. The analysis of individual sugars was made by using ion exchange chromatography and gas chromatography as described in our earlier studies (11).

**Measurement of activity of acid invertase.** The activity of acid invertase was measured by using the procedures of Long et al (15). All preparations were performed at 4 C. One-centimeter-long segments of healthy and infected leaf tissues were excised at intervals after inoculation, immersed in ice-cold ethyl acetate for 20 min and finally washed in ice-cold water. The remaining water on the leaf surface was blotted with the filter paper. Each leaf sample was transferred into a 20-ml vial containing 2 ml of 0.5 M sucrose, 2 ml of 0.1 M citrate/phosphate buffer (pH 5.6), and 6 ml of double-distilled water. The vials were incubated in a shaking water bath at 30 C. Aliquots (1 ml) were removed after 30 and 90 min. Total reducing sugars were determined by the method of Nelson (18). After incubation, leaf tissue was dried overnight at 90 C. The invertase activity was expressed as micrograms of reducing sugar per milligram dry weight per hour.

## RESULTS

**Sugar content in healthy and infected plants.** Fig. 2 shows the amounts of sucrose, glucose and fructose in healthy and infected first leaves of the susceptible cultivar Peruvian and the adult-plant-resistant cultivar Asse at intervals after inoculation with *E. g. f. sp. hordei*. Until 4 days after inoculation, the amounts of sucrose, glucose, and fructose in the infected leaves were similar to those of comparable healthy controls. As the mycelial development became visible on the infected leaves, the soluble sugars began to increase. During sporulation (6-9 days after inoculation), the amounts of sucrose, glucose, and fructose in the infected leaves were about 2-10 times more than those in comparable healthy tissues. At the early phase of infection (4 days after inoculation), there were no statistical differences in sugar content of the two cultivars tested. During colony development and sporulation, however, increases in glucose and fructose were more pronounced in Peruvian than those in Asse. Sucrose, on the other hand, accumulated in infected leaves of Asse, particularly showing a large increase at 9 days after inoculation. The increases in these sugars at the later phase of infection did not seem to be due to increases in dry matter (Table 1); i.e., sucrose content of infected leaves at 9 days after inoculation was about 6-10 times greater than those of healthy controls, whereas the dry matter of infected leaves was only about 1.3 times higher than healthy ones.

The amounts of fructose and glucose in infected third leaves at the four-leaf stage increased more in Peruvian than in Asse. However, levels of sucrose in infected leaves were greater in Asse

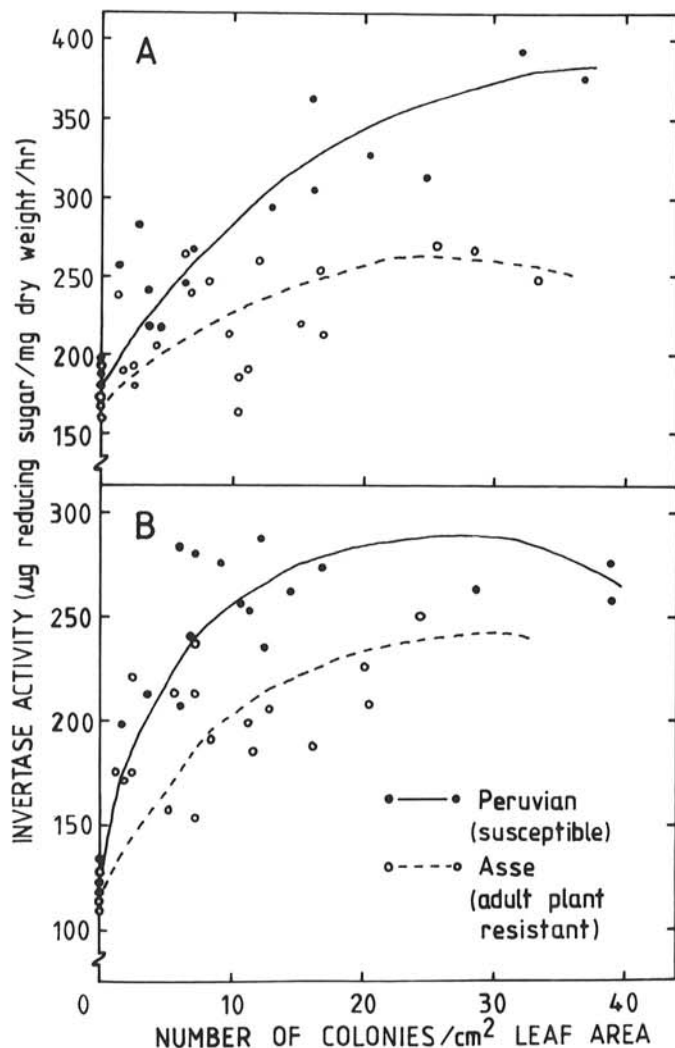


Fig. 1. Levels of invertase activity in healthy and powdery mildew infected A, first and B, third leaves of spring barley cultivars Peruvian (susceptible) and Asse (adult-plant resistant) on the 7th day after inoculation at the first leaf and fourth leaf stages, respectively. Each value represents a mean of three determinations.

than in Peruvian (Fig. 3). Sugar concentrations in noninfected fourth leaves of plants with 40–50% of the surface of the lower three leaves infected changed as compared with controls (Fig. 4). The amounts of fructose and glucose in noninfected leaves declined more in Peruvian than in Asse, whereas the amount of sucrose increased more in Asse.

**Acid invertase activity in healthy and infected plants.** The results of invertase assays from first leaves of healthy and infected plants are presented in Fig. 5. In healthy leaves, invertase activity was similar in Peruvian and Asse throughout the experiment. At 2 days after inoculation, there were no significant differences in the invertase activity in the infected leaves of the two cultivars. Infected plants showed a slightly higher activity than that of comparable healthy controls at 4 days after inoculation. However, during sporulation at 7 and 9 days, invertase activity was greater in inoculated plants of Peruvian than in Asse. At the 7th day after infection, invertase activity in the infected first and third leaves was related to the number of colonies per leaf (Fig. 1). The susceptible

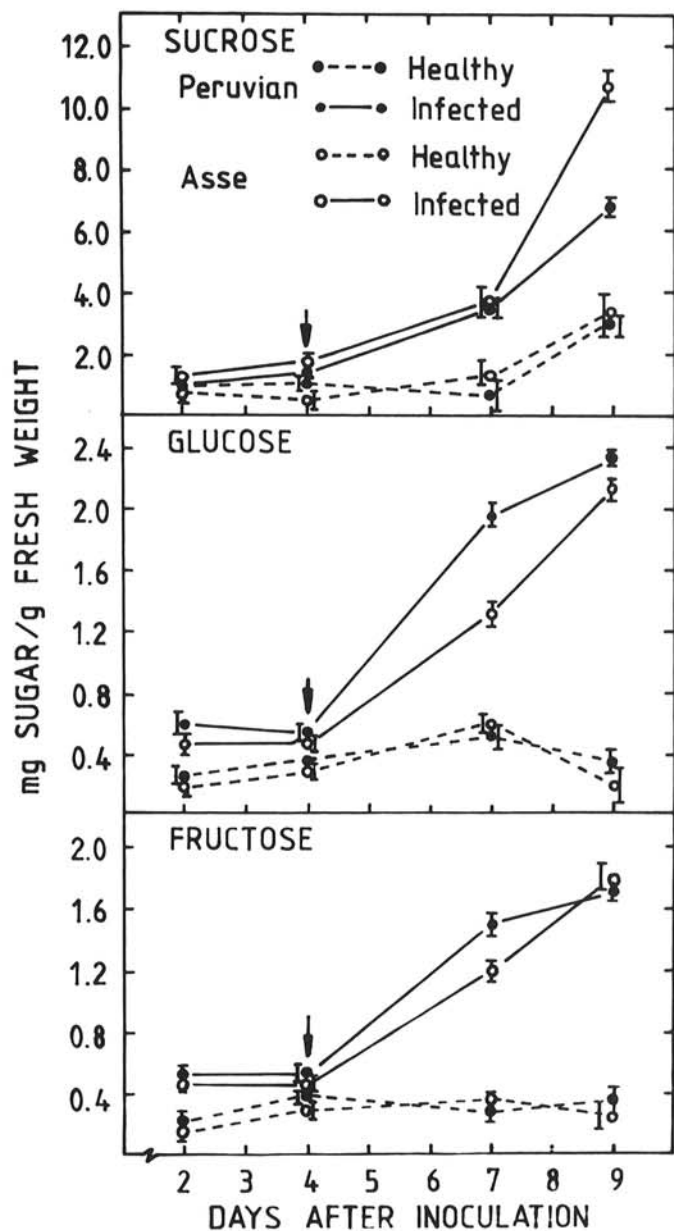


Fig. 2. Contents of sucrose, glucose, and fructose in healthy and powdery mildew infected first leaves of spring barley cultivars Peruvian (susceptible) and Asse (adult-plant resistant) at intervals after inoculation at the first leaf stage. Each value represents a mean  $\pm$  one standard deviation of three replicate samples. Arrows indicate the beginning of colony formation. The leaf areas infected were about 40% in Peruvian and 20% in Asse.

cultivar Peruvian showed higher invertase activities in infected first and third leaves at all infection intensities than did Asse.

## DISCUSSION

The experiments were done using juvenile plants of spring barley at the first leaf and fourth leaf stages, because mature plants at later growth stages could not be uniformly infected by artificial inoculation with *E. g. f. sp. hordei*. Our previous studies demonstrated that when using these inoculation methods, adult-plant resistance of spring barley to powdery mildew can be detected and estimated at the third leaf or fifth leaf stage (9,10). In contrast to a gradual decrease in colony production in the susceptible cultivar Peruvian, the adult-plant-resistant cultivar Asse showed an abrupt reduction of colony number at 7 days after inoculation as the plants became increasingly older (9). Therefore, the evaluation of the levels of soluble sugars and invertase activity in juvenile barley plants may be helpful in understanding the nutritional differences between the two cultivars, Peruvian and Asse, differing in susceptibility to powdery mildew.

The amounts of ethanol-soluble sugars such as glucose, fructose, and sucrose were enhanced in barley leaves infected by the powdery

TABLE 1. Amounts of dry matter in healthy and powdery mildew infected first leaves of spring barley cultivars Peruvian (susceptible) and Asse (adult-plant resistant) at intervals after inoculation at the first leaf stage

Cultivar	Dry matter (%) at postinoculation day <sup>a</sup>			
	2	4	7	9
Peruvian				
Healthy	8.0 $\pm$ 0.2 <sup>b</sup>	7.8 $\pm$ 0.1	7.5 $\pm$ 0.3	8.0 $\pm$ 0.2
Infected	8.2 $\pm$ 0.1	8.0 $\pm$ 0.3	8.4 $\pm$ 0.4	10.1 $\pm$ 0.4
Asse				
Healthy	9.0 $\pm$ 0.4	8.0 $\pm$ 0.2	8.3 $\pm$ 0.2	8.7 $\pm$ 0.3
Infected	8.4 $\pm$ 0.1	7.9 $\pm$ 0.3	9.4 $\pm$ 0.5	10.0 $\pm$ 0.3

<sup>a</sup> Days after inoculation of *Erysiphe graminis* f. sp. *hordei* (race C<sub>17</sub>/Amsel).  
<sup>b</sup> Each value represents a mean  $\pm$  one standard deviation of three replicate samples.

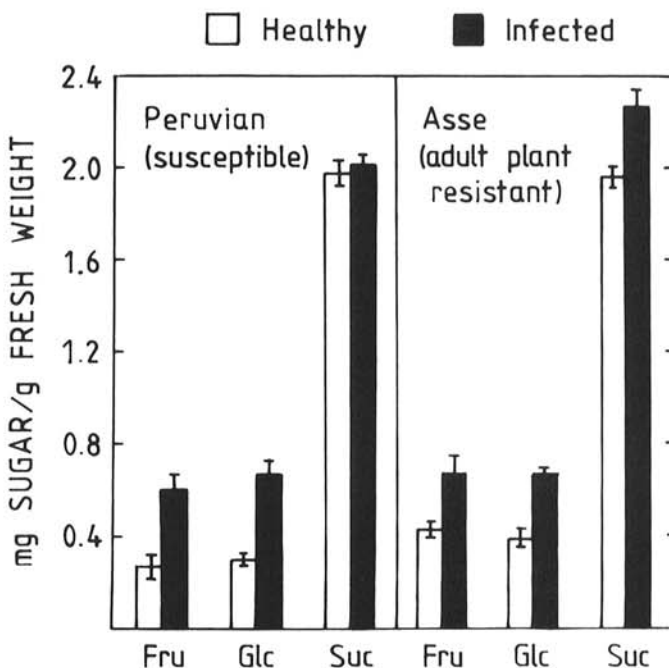


Fig. 3. Contents of fructose (Fru), glucose (Glc) and sucrose (Suc) in healthy and powdery mildew infected third leaves of spring barley cultivars Peruvian (susceptible) and Asse (adult-plant resistant) at the fourth leaf stage on the 7th day after inoculation of lower three leaves. Each value represents a mean  $\pm$  one standard deviation of three replicate samples. The leaf areas infected were about 40–50% in the two cultivars.

mildew fungus, especially during sporulation. The results obtained indicate that a rise in concentrations of these sugars may be due to their accumulation around the infection sites, reduced export from the infected leaves and stimulation of host metabolic activity favorable for the nutrient utilization by fungi (1,2,4,17,19). Contrary to the observations of Edward and Allen (7), no trehalose

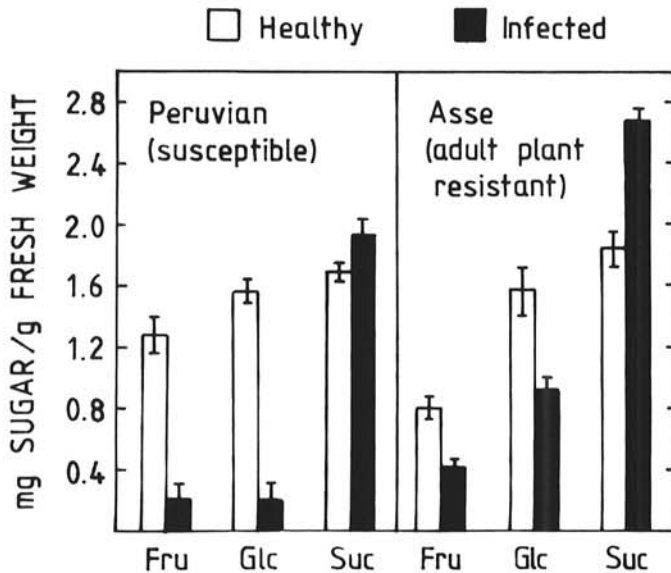


Fig. 4. Contents of fructose (Fru), glucose (Glc) and sucrose (Suc) in noninfected fourth leaves of spring barley cultivars Peruvian (susceptible) and Asse (adult-plant resistant) at the fourth leaf stage on the 7th day after inoculation of lower three leaves. Each value represents a mean  $\pm$  one standard deviation of three replicate samples. The leaf areas infected were about 40–50% in the two cultivars.

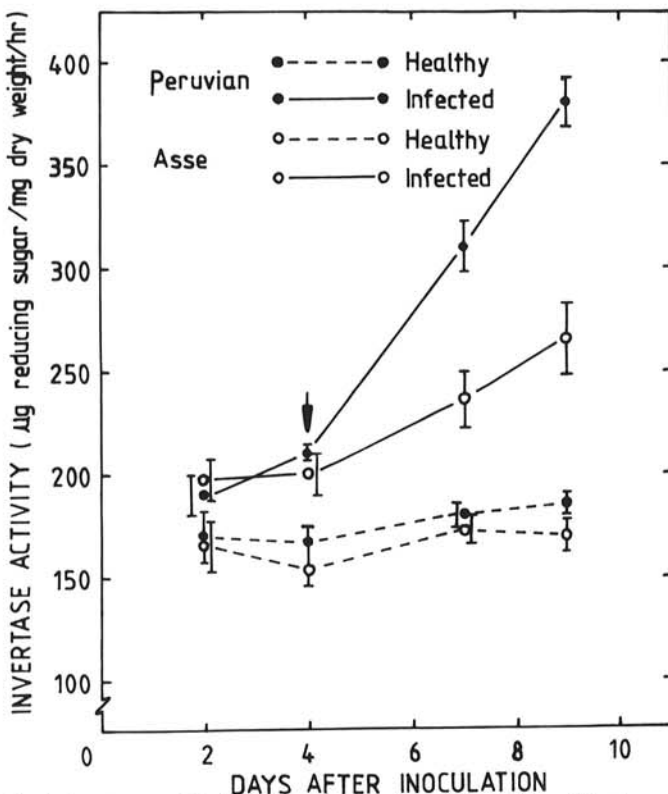


Fig. 5. Invertase activity in healthy and powdery mildew infected first leaves of spring barley cultivars Peruvian (susceptible) and Asse (adult-plant resistant) at the first leaf stage. Each value represents a mean  $\pm$  one standard deviation of three replicate samples. The leaf areas infected were about 40% in Peruvian and about 20% in Asse.

or sugar alcohols (e.g., mannitol and arabitol) could be detected by gas chromatographic analysis in healthy or powdery mildew-infected leaves.

The slight increase in soluble sugars in infected leaves at 2–4 days after infection (Fig. 2) is likely to be related to a small uptake of nutrients for the fungal developments, because the fungal structure is not yet established on the leaf surface. There were no differences in amounts of sugars between the two cultivars at 4 days after infection. During sporulation, there was an increase of glucose and fructose in both cultivars, but it was more pronounced in Peruvian than in Asse. This could explain stimulated metabolic activities favorable for the rapid conversion of host sugars into metabolites unique to the powdery mildew fungus, especially in the susceptible cultivar Peruvian.

The observed increase in activity of acid invertase in infected leaves of Peruvian and Asse is in agreement with observations of workers studying other host-pathogen systems (3,15,16). Mitchell et al (17) have demonstrated that in wheat leaves infected by *Puccinia graminis* var. *tritici*, there were increases in glucose and fructose during sporulation at a high infection intensity that may be due to stimulated acid invertase activity. Recently, Clancy and Coffey (4) failed to detect increased acid invertase activity in resistant flax infected with *Melampsora lini*. Our findings that there is a greater rise of acid invertase activity in the susceptible cultivar than in the adult-plant-resistant cultivar may suggest a causal relationship between rapid fungal development and accelerated carbohydrate catabolism in susceptible cultivars.

An interesting fact is that the amount of sucrose in upper noninfected leaves of barley plants with lower leaves infected was enhanced in comparison to those of comparable healthy plants, whereas glucose and fructose declined markedly (Fig. 4). These data suggest that sucrose may be mobilized in noninfected leaves to transport into infected leaves functioning as an active sink. In particular, the marked decrease in amounts of glucose and fructose in the susceptible cultivar Peruvian could be due to the greater exhaustion of the sugars required for an enhanced movement of sucrose into infected lower leaves.

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