# Effect of Energy-Requiring Defense Reactions on Yield and Grain Quality in a Powdery Mildew-Resistant Barley Cultivar

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## ABSTRACT

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Respiration and yield were measured in plants of barley cultivar Sultan following inoculations with two races of *Erysiphe graminis* f. sp. *hordei* (the powdery mildew pathogen), one avirulent and the other virulent on Sultan. Respiration measurements demonstrated that plants responding resistant to inoculation with the avirulent race had an 80% increase in the rate of respiration starting within 16–24 hr after inoculation and returning to the normal level at the time when respiration started to increase in plants inoculated with the virulent race. Although plants remained entirely free of symptoms after inoculation with an avirulent race, the grain yield was significantly reduced by 7%, and the kernel weight by 4%. The content of grain protein was reduced from 9.75 to 9.38%, equivalent to an 11% reduction in the yield of grain protein. In susceptible, infected plants the grain yield was reduced by 26%, and the kernel weight by 11%. The protein content of grain was reduced from 9.75 to 9.25%, which is equivalent to a reduction in the yield of grain protein by 30%. The results indicate that in inoculated, highly mildew-resistant barley plants there was an increased requirement of host energy, probably due to enhanced biochemical activities involving active defense reactions. The greatly increased respiratory rate deprived the resistant host of energy available for growth, and led to a reduction in grain yield and grain quality, although no visible symptoms appeared.

Additional key words: *Erysiphe graminis* f. sp. *hordei*, protein yield, resistance.

In incompatible combinations between barley (*Hordeum vulgare* L.) and the powdery mildew fungus (*Erysiphe graminis* DC. ex Mérat f.sp. *hordei* Marchal) resistant plants react to inoculation with an increased biosynthetic activity (7,9,11,12) and changes in the rate of respiration (2,10,14,15,17).

Whether the increased rates of biosynthetic and respiratory activities are causally related had not been determined.

Since any rise in biochemical activities requires energy, such rises may influence the energy status of the host. Comparing the respiratory rates of compatible and incompatible host-pathogen interactions, Smedegaard-Petersen (17) found that hypersensitivity is associated with a rapid, temporary increase in respiration of resistant plants. The increase started 16–24 hr after inoculation and returned to the normal level after a few days. In susceptible plants, however, respiration started to increase later and remained at a high level until the beginning of senescence. He suggested that disease resistance based on incompatibility between host and pathogen is associated with energy-requiring processes (probably involving biochemical defense reactions) which deprive the host of energy and ultimately lead to a lower yield.

The object of the present experiments was, therefore, to investigate whether there is a higher demand for energy in barley plants inoculated with an avirulent race of *E. graminis* (resistant reaction) than in uninoculated barley plants and to determine the possible consequences for plant growth and yield.

## MATERIALS AND METHODS

The two-rowed spring barley cultivar Sultan was used in all experiments. The resistance of this cultivar against powdery mildew is conditioned by a single dominant or semidominant gene derived from cultivar Arabische (19,21).

Two single-spore cultures of *Erysiphe graminis* f.sp. *hordei* were used. Race 15-0 was avirulent on cultivar Sultan, eliciting reactions of the type 0 (highly incompatible interaction without visible symptoms). Isolate 1-4 was virulent on Sultan and elicited type 4

reactions (fully compatible interaction). The two cultures were maintained on plants of barley cultivar Proctor which is fully susceptible (type 4) to both.

For use in respiration experiments, barley seedlings were grown in a greenhouse maintained at a temperature of approximately 18 C and the normal day-night light cycle for May. Seedlings were inoculated with conidia of *E. graminis* when the first two leaves were fully expanded. The procedures for inoculation, incubation, and determination of oxygen uptake in the dark by using standard manometric techniques were previously described (16,17). Respiration was calculated as microliters of oxygen uptake per 10 mg dry weight of leaf tissue per hour.

Yield experiments were carried out in three growth chambers (Canadian PGW, 12 m²). The plants were grown in plastic pots 30 cm in diameter, 35 cm high, and filled with 9 kg of soil. The pots were set in plastic containers, 35 cm in diameter and 10 cm high. Watering was carried out by applying water to the lower container. A total of 20 seeds was sown in each container; after emergence the seedlings were thinned to 15 plants per pot. Fertilizer was applied at intervals up to heading. The experiments were repeated once.

Immediately after seed sowing, the containers were placed in the growth chambers under fluorescent light with an intensity of 1,950 W/m² and a day-night cycle of 18 hr of light and 6 hr of darkness. The chambers were adjusted to a temperature of $18 \pm 1$ C in the light period and $14 \pm 1$ C in the dark period. As the plants approached maturity, until harvest, the temperature was raised to 22 C.

To ensure similar conditions in the three growth chambers, light, temperature, and humidity were automatically controlled and continuously recorded by automatic recorders during the entire growth period. Also, watering and application of fertilizer were carried out as uniformly as possible.

From the five-leaf stage until immediately after heading, plants in two of the chambers were continually inoculated with conidia of the avirulent race 15-0, or the virulent race 1-4, respectively, whereas plants in the third chamber were not inoculated. Inoculation was carried out by placing three pots with susceptible, infected plants (cultivar Proctor) on elevated columns in each chamber. Once a week the pots were replaced with new infected

plants to ensure a continuous source of inoculum.

Fully ripened plants were harvested and the following characters were measured: 1, height in centimeters (from the soil line to the top of the spike exclusive of the awns); 2, number of tillers per plant; 3, number of seed-bearing spikes per plant. (For characters 1 to 3, average for each pot was calculated); 4, grain and straw dry matter yield in gram per pot was obtained after drying the grain and straw at 100 C for 24 hr; 5, number of kernels was obtained by counting the number of kernels per pot; and 6, kernel weight which was obtained by dividing the grain weight by the number of grains.

Nitrogen content in grain and straw was determined on three samples from each pot and carried out on an autoanalyser (Technicon) according to the micro-Kjeldahl procedure described by Varley (20). The N-content was multiplied by 6.25 to give percent protein content. Statistical analyses were carried out according to procedure outlined by Steel and Torrie (18).

## RESULTS

**Respiration of inoculated barley leaves.** Figure 1 shows the respiratory rate in a typical experiment with the barley cultivar Sultan, in the two-leaf stage, inoculated with the two races 15-0 and 1-4 of *E. graminis*, the former avirulent and the latter virulent on cultivar Sultan. The resistant leaves reacted to inoculation with a temporary sharp increase in oxygen uptake starting within 16 hr after inoculation. Twenty-four hours after inoculation it reached a maximum which was approximately 80% higher than that of the uninoculated controls. After that time it quickly decreased to about the level of uninoculated controls, at about the same time as respiration began to increase in susceptible inoculated plants.

In some experiments, in which the plants were inoculated at the one-leaf stage, the maximum respiratory rate did not occur until 48 hr after inoculation, but in all cases it decreased to the normal level about the same time as respiration began to increase in susceptible inoculated plants.

The results are consistent with previous results (17) obtained with other host-pathogen interactions and indicate that plant disease resistance based on incompatibility between host and pathogen is associated with an increased consumption of host energy.

**Yield of inoculated barley plants.** The uninoculated plants in chamber I grew vigorously during the whole growth period and did not show any sign of contamination from external inoculum. No mildew infections were observed on plants in chamber II, which were continually inoculated with the avirulent mildew race 15-0, and the plants remained entirely free of disease symptoms (Fig. 2). There was a tendency, however, for leaves to become senescent earlier than in the uninoculated control plants.

Although the resistant plants did not show disease symptoms, the grain yield was significantly reduced by 7% and the kernel weight by 4%. The protein content of grain was reduced from 9.75 to 9.38% of the grain weight (Table 1). This is equivalent to a reduction in the average yield of grain protein per pot by 11% (Table 2). The straw yield of resistant plants was reduced by 3% and the straw length by 5.7 cm, compared with the uninoculated controls (Table 3).
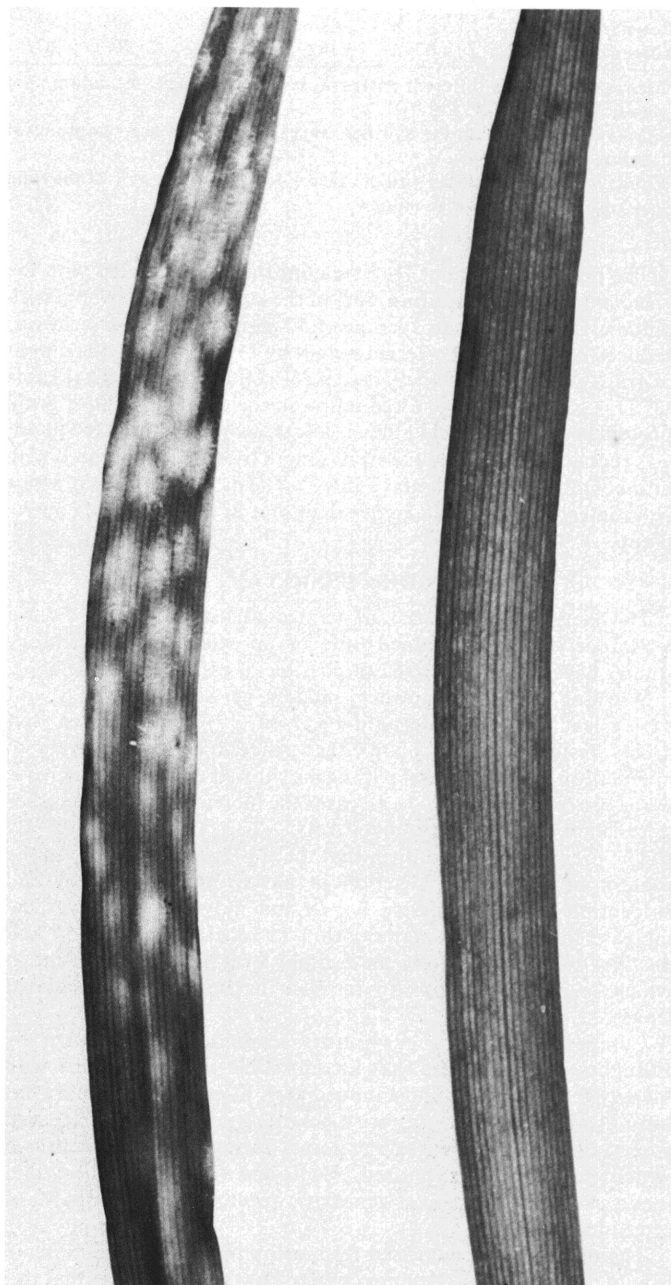


**Fig. 2.** Leaves of the barley cultivar Sultan inoculated with an avirulent race (resistant reaction) and a virulent race (susceptible reaction) of *E. graminis* f. sp. *hordei*.
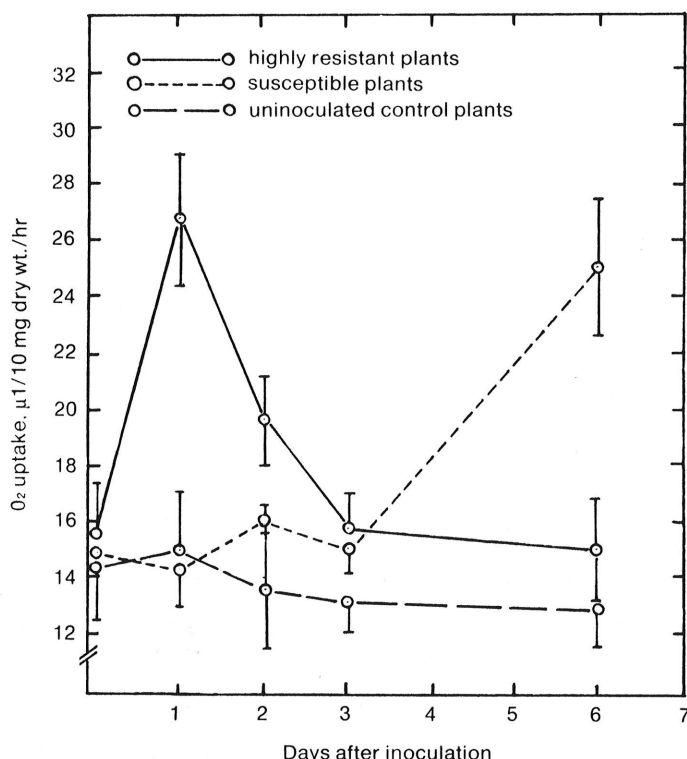


**Fig. 1.** Respiration of cultivar Sultan barley leaves resistant and susceptible to inoculation with *Erysiphe graminis* f. sp. *hordei* races 15-0 (avirulent) and 1-4 (virulent), respectively. The second leaf of each plant used. Vertical bars represent the standard deviation.

TABLE 1. Yield of grain, number of spikes per plant, number of kernels per spike, kernel weight, and percent protein in grain of plants of barley cultivar Sultan inoculated with a virulent or an avirulent isolate of *E. graminis* f.sp. *hordei*

| Host reaction | Yield of grain | | Spikes per plant (no.) | Kernels per spike (no.) | Kernel weight | | Protein in grain (%) |
| | Grams per pot | Percent of control | | | mg | Percent of control | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Uninoculated control | 66.9$^x$ a | 100 | 5.8 a | 21.5 a | 42.4 a | 100 | 9.75 a |
| Resistant$^y$ | 62.0 b | 93 | 5.5 b | 21.1 b | 40.5 b | 96 | 9.38 b |
| Susceptible$^z$ | 49.2 c | 74 | 5.3 c | 19.5 c | 37.6 c | 89 | 9.25 c |

$^x$Means followed by different letter (a, b, and c) in the same column are significantly different, $P = 0.01$.
$^y$Plants inoculated with the avirulent race 15-0. No disease symptoms at heading.
$^z$Plants inoculated with the virulent race 1-4. At heading, 56% of the total leaf area was covered with mildew.

TABLE 2. Yield of protein of plants of the barley cultivar Sultan inoculated with a virulent or an avirulent race of *E. graminis* f. sp. *hordei*

| Host reaction | Yield of protein (grams per pot) | | | Yield of protein (% of control) | |
| | Grain | Straw | Total | Grain | Total |
| --- | --- | --- | --- | --- | --- |
| Uninoculated control | 6.52$^x$ a | 2.59 a | 9.11 a | 100 | 100 |
| Resistant$^y$ | 5.82 b | 2.48 b | 8.30 b | 89 | 91 |
| Susceptible$^z$ | 4.55 c | 3.36 c | 7.91 c | 70 | 87 |

$^x$Means followed by different letters (a, b, and c) in the same column are significantly different, $P = 0.01$.
$^y$Plants inoculated with the avirulent race 15-0. No disease symptoms at heading.
$^z$Plants inoculated with the virulent race 1-4. At heading, 56% of the total leaf area was covered with mildew.

The plants in chamber III were continually inoculated with the virulent race 1-4. At heading, 56% of the leaf area was covered with mildew, measured as an average of 50 plants. The grain yield was reduced by 26% and the kernel weight by 11%. The protein content of grain was reduced from 9.75 to 9.25% of the grain weight (Table 1). This is equivalent to a reduction in the average yield of grain protein per pot by 30% (Table 2). The straw yield of infected plants was reduced by 12% and the straw length by 4.9 cm compared with uninoculated control plants (Table 3). The protein content of straw from infected plants was higher than that in uninoculated control plants.

## DISCUSSION

The energy status of resistant plants is an aspect of plant disease resistance that has obtained little or no attention. Respiratory studies have not been related to possible consequence in yield.

Working with barley powdery mildew, net blotch of barley, and barley leaf stripe, Smedegaard-Petersen (17) demonstrated that highly resistant barley plants that reacted hypersensitively to inoculation with the causal pathogens showed up to 110% increase in the rate of respiration. In all cases the increases occurred earlier in resistant than in susceptible plants.

The present results confirm that there is clear difference in the pattern of respiratory reaction in barley plants resistant and susceptible to *E. graminis* f.sp. *hordei*. In highly resistant leaves, the increase in respiration starts within 16 hr after inoculation and declines to approximately the normal level at 72 hr, the time at which respiration begins to increase in susceptible inoculated leaves.

Comparing the early respiratory response with the data by Ellingboe (5) it appears that incompatible tissue responds with increased respiration almost immediately after incompatibility has been established. Working with powdery mildews of barley and wheat, Ellingboe (5) defined the points during primary infection at which resistance is expressed. He found that the earliest that incompatible parasite-host genotypes reacted was at about 12 hr after inoculation.

The return of the increased respiration in resistant reactions to that of the uninoculated controls after a few days indicates that this temporary rise is not part of an irreversible disease process. This may be the case, however, in susceptible infected tissue in which the respiration begins to increase at the same time as the visible disease symptoms begin to develop, and then remains at a high level until the beginning of senescence.

To explain the obvious need for additional respiratory energy in resistant plants, it should be stressed that resistance is associated with a series of structural and biochemical events in the host plant, including syntheses of antifungal compounds (12,13), increased syntheses of nucleic acids (11), accumulation of new, aromatic substances (9), increased peroxidase and ethylene production (7), and changes in the content of carotenoids (6).

Although the role of such activities in resistance is a matter of much debate, there is good experimental evidence to suggest that at least some of them are involved in active defense reactions. Thus, Ouchi et al (13) demonstrated that barley plants inoculated with an avirulent race of *E. graminis* became partly resistant to a race originally virulent on that plant. The resistance conferred by the primary infection with an incompatible race indicated the involvement of phytoalexins. Accordingly Oku et al (12) detected high phytoalexin activity 12–20 hr after inoculation in an incompatible host-parasite combination. In compatible combinations, no phytoalexin formation was found at this stage of infection, although antifungal activity also occurred in susceptible hosts at a later stage.

Hence, in highly mildew-resistant barley plants, increased respiration and increased biosynthetic activities occur simultaneously 12–24 hr after inoculation. Our results indicate that the enhanced respiration in mildew-resistant barley plants is due to energy-requiring defense reactions. The result is a drain on the host energy which leads to reductions in grain yield and grain quality. This conclusion is supported by the observation that resistant, inoculated plants tend to senesce earlier.

It is well known that increases in oxygen uptake do not necessarily result in the production of energy. Breakdown of cellular compartmentation and other kinds of tissue disintegration may lead to pathological functions of enzymes such as peroxidases, polyphenol oxidases, and ascorbic acid oxidases that contribute to the oxygen uptake. However, as pointed out by Daly (4), these kinds of reactions are most prominent during cellular degradation in the later stages of infection and it is unlikely that such reactions can account for the marked increase in oxygen uptake during the initial stages of infection.

The question also may arise as to how much the pathogen itself contributes to the overall respiration. Results of the classic works by Allen and Goddard (1) and Millerd and Scott (10) showed that barley tissue with the mildew fungus removed respires at almost the same rate as did tissue with the fungus attached. In our experiments the marked differences in oxygen uptake by incompatible and compatible interactions during the first 72 hr after inoculation clearly indicate that the fungus does not contribute significantly during this first period of infection.

The inoculum pressure is important in the evaluation of the yield reduction in susceptible and resistant plants. As previously mentioned, an equal amount of inoculum of the two races was applied to plants in the growth chambers. A measure of the inoculum density appears from the fact that 56% of the total leaf area in susceptible plants was covered with mildew at heading.

The fact that an avirulent race of powdery mildew is able to cause significant reductions in grain yield and grain quality without causing visible symptoms involves some interesting practical

TABLE 3. Yield of straw, length of straw, number of tillers per plant, and percent protein in straw of plants of barley cultivar Sultan inoculated with a virulent or an avirulent race of *E. graminis* f. sp. *hordei*

| Host reaction | Yield of straw | | Length of straw (cm) | Tillers per plant (no.) | Protein in straw (%) |
|---|---|---|---|---|---|
| | Grams per pot | Percent of control | | | |
| Uninoculated control | 78.2[x] a | 100 | 107.8 a | 6.2 a | 3.31 a |
| Resistant[y] | 76.2 b | 97 | 102.1 b | 6.2 a | 3.25 a |
| Susceptible[z] | 68.9 c | 88 | 102.9 c | 5.6 b | 4.88 b |

[x]Means followed by different letters (a, b, and c) in the same column are significantly different, $P = 0.01$.
[y]Plants inoculated with the avirulent race 15-0. No disease at heading.
[z]Plants inoculated with the virulent race 1-4. At heading, 56% of the total leaf area was covered with mildew.

aspects. Under field conditions it is evident that a certain high concentration of avirulent *E. graminis* conidia must be present in order to cause measurable yield reduction, and such high concentrations of spores usually may not be present. However, studies in progress (Smedegaard-Petersen, *unpublished*) indicate that a number of nonpathogenic leaf surface fungi are able to bring about reactions much like those caused by avirulent *E. graminis* conidia. That the fungal flora of the leaf surface also affect the yield is indicated by several years of field experiments showing that removal of the fungal flora from the leaf surfaces with different fungicides increased the grain yield significantly, although virtually no disease symptoms could be detected in the untreated control plants (Smedegaard-Petersen, *unpublished*).

Another important aspect of the results is that the protein content of grain was significantly reduced, not only in mildew-infected plants, but also in highly resistant barley plants inoculated with *E. graminis*. As far as we are aware this is the first report showing that incompatibility may influence the protein content of the resistant host. In infected plants, however, similar results have been demonstrated. Johnson et al (8) found that protein percentages in flour made from grain produced on mildewed wheat plants was negatively correlated with percent mildew on the leaves. Also, Caldwell et al (3) found that heavy infection with leaf rust uredospores (*Puccinia recondita tritici*) reduced the protein content in winter wheat grain. Normally it is found that decreased yield and kernel weight result in a higher percent protein content of the grain.

### LITERATURE CITED

1. Allen, P. J., and Goddard, D. R. 1938. A respiratory study of powdery mildew of wheat. Am. J. Bot. 25:613-621.
2. Antonelli, E., and Daly, J. M. 1966. Decarboxylation of indoleacetic acid by near-isogenic lines of wheat resistant or susceptible to *Puccinia graminis* f. sp. *tritici*. Phytopathology 56:610-618.
3. Caldwell, R. M., Kraybill, H. R., Sullivan, J. T., and Compton, L. E. 1934. Effect of leaf rust (*Puccinia triticina*) on yield, physical characters, and composition of winter wheats. J. Agric. Res. 48:1049-1071.
4. Daly, J. M. 1976. The carbon balance of diseased plants: Changes in respiration, photosynthesis and translocation. Pages 450-479 in: R. Heitefuss and P. H. Williams, eds. Physiological Plant Pathology. Springer-Verlag, Berlin, Germany.
5. Ellingboe, A. H. 1972. Genetics and physiology of primary infection by *Erysiphe graminis*. Phytopathology 62:401-406.
6. Haspel-Horvatovic, E., and Paulech, C. 1969. Changes of oxidized and reduced carotenoids, of the respiration and the photosynthesis of barley (*Hordeum sativum* L.) in connection with its resistance against powdery mildew. Pages 396-401 in: H. Metzner, ed. Progress in Photosynthesis Research. Tübingen, Germany.
7. Hislop, E. C., and Stahmann, M. A. 1971. Peroxidase and ethylene production by barley leaves infected with *Erysiphe graminis* f. sp. *hordei*. Physiol. Plant Pathol. 1:297-312.
8. Johnson, J. W., Baenziger, P. S., Yamazaki, W. T., and Smith, R. T. 1979. Effects of powdery mildew on yield and quality of isogenic lines of "Chancellor" wheat. Crop Sci. 19:349-352.
9. Mayama, S., and Shishiyama, J. 1978. Localized accumulation of fluorescent and u.v.-absorbing compounds at penetration sites in barley leaves infected with *Erysiphe graminis hordei*. Physiol. Plant Pathol. 13:347-354.
10. Millerd, A., and Scott, K. 1956. Host pathogen relations in powdery mildew of barley. II. Changes in respiratory pattern. Aust. J. Biol. Sci. 9:37-44.
11. Oku, H., Ouchi, S., and Sato, M. 1973. Nucleic acid metabolism in powdery mildew-infected barley leaves as associated with symptom development. Rep. Tottori Mycol. Inst., Jpn. 10:511-516.
12. Oku, H., Ouchi, S., Shiraishi, T., Komoto, Y., and Oki, K. 1975. Phytoalexin activity in barley powdery mildew. Ann. Phytopathol. Soc. Jpn. 41:185-191.
13. Ouchi, S., Oku, H., Hibino, C., and Akiyama, I. 1974. Induction of accessibility and resistance in leaves of barley by some races of *Erysiphe graminis*. Phytopathol. Z. 79:24-34.
14. Paulech, C. 1967. Einfluss des Pilzes *Erysiphe graminis* f.sp. *hordei* Marchal auf die Respiration anfälliger und resistenter Gerstensorten (*Hordeum sativum* L.). Biologia 22:202-209.
15. Scott, K. J., and Smillie, R. M. 1966. Metabolic regulation in diseased leaves. I. The respiratory rise in barley leaves infected with powdery mildew. Plant Physiol. 41:289-297.
16. Smedegaard-Petersen, V. 1977. Respiratory changes of barley leaves infected with *Pyrenophora teres* or affected by isolated toxins of this fungus. Physiol. Plant Pathol. 10:213-220.
17. Smedegaard-Petersen, V. 1980. Increased demand for respiratory energy of barley leaves reacting hypersensitively against *Erysiphe graminis*, *Pyrenophora teres* and *Pyrenophora graminea*. Phytopathol. Z. 99:54-62.
18. Steel, R. G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York. 481 pp.
19. Torp. J., Jensen, H. P., and Helms-Jørgensen, J. 1978. Powdery mildew resistance genes in 106 northwest European spring barley varieties. Pages 75-102 in: Royal Veterinary and Agricultural University Yearbook, Copenhagen, Denmark.
20. Varley, J. A. 1966. Automatic methods for the determination of nitrogen, phosphorus and potassium in plant material. Analyst. 91:119-126.
21. Wolfe, M. S. 1972. The genetics of barley mildew. Rev. Plant Pathol. 51:507-522.