Research

# Effect of the *Lr9* Resistance Gene on Pathogenesis of the Wheat Leaf Rust Fungus

**S. Ortelli, U. Giezendanner,** and **J. Nösberger,** Swiss Federal Institute of Technology, Institut für Pflanzenwissenschaften, ETH-Zentrum, CH-8092 Zürich; and **H. Winzeler, B. Keller,** and **M. Winzeler,** Swiss Federal Research Station for Agronomy, CH-8046 Zürich-Reckenholz

**ABSTRACT**

Ortelli, S., Giezendanner, U., Nösberger, J., Winzeler, H., Keller, B., and Winzeler, M. 1996. Effect of the *Lr9* resistance gene on pathogenesis of the wheat leaf rust fungus. Plant Dis. 80:14-18.

The pathogenesis of two leaf rust fungus isolates on seedlings of the susceptible cultivar Arina and two of its resistant near-isogenic lines (NILs) carrying *Lr9* was studied by means of epifluorescence microscopy. The orientation of urediniospore germ tubes and differentiation of appressoria over stomata were similar on susceptible and resistant plants. The development of the fungus in the susceptible host was not stopped by any detectable host resistance reaction, and the number of haustorial mother cells increased exponentially. Fungal sporulation took place 7 to 8 days after inoculation. Host cells of the resistant *Lr9* lines close to the haustorial mother cells died 24 to 44 h after inoculation due to a hypersensitive reaction. Before ceasing its growth, the fungus succeeded in forming generally only 1 to 3 haustorial mother cells. The necrotic area around an infection site reached an average area of 10,000 μm$^2$. The potential influence of this defense reaction on yield is discussed.

**ZUSAMMENFASSUNG**

Der Infektionsverlauf von Braunrost wurde auf der anfälligen Sorte Arina und zwei braunrostresistenten, nah isogenen Linien (NILs) mit dem Resistenzgen *Lr9* untersucht. Keimpflanzen wurden mit zwei Braunrostisolaten inokuliert, die auf *Lr9* avirulent waren. Der Infektionsverlauf und die Abwehrreaktionen wurden mit Hilfe der Epifluoreszenzmikroskopie beobachtet. Die Orientierung der Urediniosporen-Keimschläuche und die Differenzierung des Appressoriums über den Spaltöffnungen war bei den anfälligen und resistenten Pflanzen vergleichbar. Die weitere Entwicklung des Braunrostes wurde auf dem anfälligen Wirt durch keine Abwehrreaktion gehemmt. Der Zuwachs der Haustorienmutterzellen (HMC) verlief exponentiell. Die Sporulation des Pilzes setzte 7 bis 8 Tage nach der Inokulation ein. Auf den resistenten NILs starben die Wirtszellen in der Nähe der ersten Haustorienmutterzellen als Folge einer hypersensitiven Reaktion 24 bis 44 Stunden nach der Inokulation ab. Bevor das Wachstum des Pilzes zum Stillstand kam wurden meist nur eine bis drei HMC pro Infektionsstelle gebildet. Die nekrotisierte Fläche betrug pro Infektionsstelle im Durchschnitt 10,000 μm$^2$. Der Einfluss dieser Abwehrreaktion auf die Ertragsbildung wird diskutiert.

*Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici*, the causal agent of wheat leaf rust, is considered to be one of the most devastating pathogens of wheat (*Triticum aestivum* L.). Therefore, leaf rust resistance is a major goal in most wheat breeding programs. At the Swiss Federal Research Station for Agronomy (FAP), a backcross program was carried out to transfer the leaf rust resistance gene *Lr9* (13) into the genetic background of Arina (5). At present, Arina is the most important bread wheat cultivar in Switzerland. Leaf rust–resistant lines selected in this program were morphologically very close to Arina. However, the yield of these lines was on average lower than for Arina in fungicide-treated experiments (15); also, in untreated trials, the resistant *Lr9* lines did not outyield the susceptible recurrent parent (5; *unpublished data*). A research project was initiated to study the relationship between *Lr9* and this yield reduction. In the present study, the effect of *Lr9* on the pathogenesis of leaf rust was studied in order to identify reactions that can be related to reduced yield. The pathogenesis of wheat leaf rust is quite complex. On the leaf surface, spores germinate overnight under high humidity with an optimal temperature range between 10 and 22°C (11). Response to the topography of the leaf surface directs the fungal germ tube to grow perpendicularly to the venation of the leaf to the nearest stoma (1,9). There, the apex of the germ tube forms an appressorium. After the penetration of the stoma, a substomatal vesicle is developed from which the primary hypha invades the intercellular space. It produces haustorial mother cells that develop haustoria in the host cells. Secondary hyphae are formed from the haustorial mother cells and grow in the intercellular space, and these hyphae produce new haustorial mother cells and haustoria. In a susceptible host, the number of haustoria produced within each colony increases exponentially. Given optimal conditions, the fungus undergoes reproduction within a few days, and the typical symptoms become visible at the leaf surface as sporulating pustules.

Resistance to rusts can potentially interfere in several crucial steps of this complex process of infection. Dealing with an appropriate classification of the different factors and mechanisms of resistance, Hart (7) proposed to distinguish among morphological, physiological, and functional resistance. Each of these kinds of resistance might interfere negatively with the yield physiology of the cereal plant, for example through disadvantages due to morphological changes of the leaf surface or to altered metabolic activity.

In the present work, race-specific leaf rust resistance was investigated in the model system of isogenic lines generated from Arina and carrying the resistance gene *Lr9*. The aim of the work was to recognize resistance mechanisms of either a morphological, physiological, or functional type. Seedlings of the cultivar Arina and two leaf rust–resistant near-isogenic lines were microscopically analyzed after inoculation. A variety of factors responsible for host resistance to the parasite is discussed in relation to their interference with the yield of the cereal plant.

## MATERIALS AND METHODS

**Plant material.** The leaf rust–susceptible Swiss cultivar Arina and two of its resistant, near-isogenic lines (NILs) carrying *Lr9* were chosen for the experiment. The two NILs (*Lr9*-A and *Lr9*-B) were developed from two independent backcross populations with Arina as a recurrent parent. The *Lr9* gene donor was R.L. 6010 (Transfer/6*Tc). The NILs *Lr9*-A and *Lr9*-B were F8 lines derived from the seventh backcross (R.L. 6010/7*Arina).

Accepted for publication 10 July 1995.

Publication no. D-1995-1202-09R
© 1996 The American Phytopathological Society

**Growing conditions**. Twenty seeds were germinated and grown in pots filled with soil (height 8 cm, diameter 6 cm) in a growth chamber. To avoid unwanted infections, the pots were protected with cellophane bags. During the whole experiment, the growing plants were provided with a fertilizer solution containing Lonzin (160 g of N, 80 g of $P_2O_5$, and 240 g of $K_2O$ $kg^{-1}$; Lonza, Basel, Switzerland) at 1 g $liter^{-1}$. The day/night temperature was 20/16°C, and the relative day/night humidity was 85/99%. The length of the photoperiod was 16 h at a photosynthetic photon flux density of 360 $\mu E \cdot m^{-2} \cdot s^{-1}$, with a 1-h transition interval both in the morning and in the evening.

**Pathogen and inoculation**. Two leaf rust isolates (89-035 and 90-039) collected in Switzerland were used in the inoculation experiments. Previous experiments showed that they were avirulent on R.L. 6010, *Lr9*-A, and *Lr9*-B. Both isolates were multiplied on the susceptible cultivar Moléson. Spores were desiccated in a desiccator and either stored in plastic vials at −80°C or used directly as inoculum in the experiments. To rehydrate the spores, the vials were stored in petri dishes containing a wet filter paper for a few hours before inoculation. Primary leaves of the seedlings were infected 10 days after sowing. The cellophane bags were removed, and leaves were sprayed with a spore suspension prepared with Soltrol 170 (Phillips Petroleum, Paris, France) as the carrying medium. To achieve a uniform inoculation, plants were put on a constantly rotating plate during spraying. Oil droplets from the spore-carrying medium were evaporated by putting the plants in a well-ventilated area for 1 h. Finally, the pots were placed in a plastic tray (20 × 20 × 4 cm) under a spore-tight hood.

**Sample preparation**. Primary leaves were harvested at 6, 12, 24, 44, 72, 96, 120, 144, and 192 h after inoculation and prepared for fluorescence microscopy. Sample preparation was performed using a combination of methods suggested by Rohringer et al. (16) and Kuck et al. (12). Leaves were soaked for 2 h at 45°C in a solution containing 70% chloroform and 30% methanol. Leaves were bleached during 4 h at 55°C in a lactophenol/ethanol solution (1:2) until completely discolored. After a short double rinse in the chloroform/methanol solution, leaves were stained in a solution containing 70% chloroform, 29.9% methanol, and 0.1% Leucophor BMB (Sandoz, Basel, Switzer-

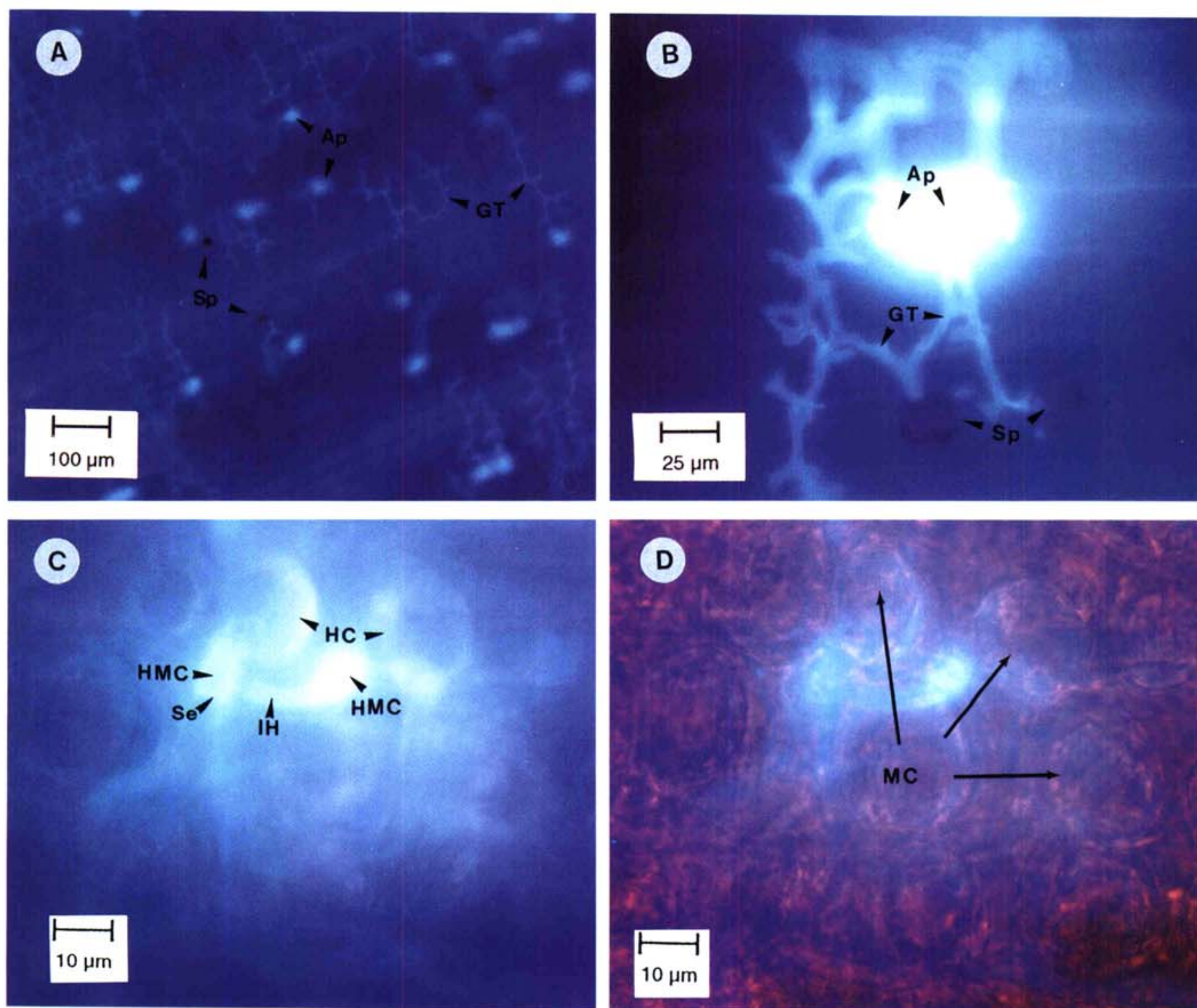


**Fig. 1.** Epifluorescence photomicrographs showing crucial steps in the infection process of *Puccinia recondita* f. sp. *tritici* on primary wheat leaves: **(A and B)** superficial growth and **(C and D)** intercellular growth events. **(A)** Directional growth of the germ tube and appressorium differentiation on leaf rust–resistant line *Lr9*-B, 18 h after inoculation. **(B)** Urediniospore germination, germ tube branching, and appressorium differentiation on the leaf rust–susceptible cultivar Arina, 96 h after inoculation. **(C)** The haustorial mother cell is (shown here 96 h after inoculation) clearly separated from the hypha by a septum. **(D)** The haustorium colonizes a mesophyll cell. The haustorial mother cell generates a secondary hypha able to generate another haustorial mother cell with an associated haustorium. Ap = appressorium, GT = germ tube, HC = host cell colonized by a haustorium, HMC = haustorial mother cell, IH = intercellular hypha, MC = mesophyll cell, Se = septum, and Sp = spore.

land) for 30 s and again rinsed three times in the chloroform/methanol solution. The samples were mounted on slides and stored in an aqueous solution containing 50% glycerol and a trace of lactophenol as preservative. This staining technique allows the simultaneous visualization of fungal development and host tissue necrosis, but optimal resolution of haustoria was not always possible.

**Microscopy**. The samples were analyzed using a Diaphot microscope by Nikon supplied with an epifluorescence device. A permanent light source of 365 nm, UV 330 to 380 filters, a dichroic mir-

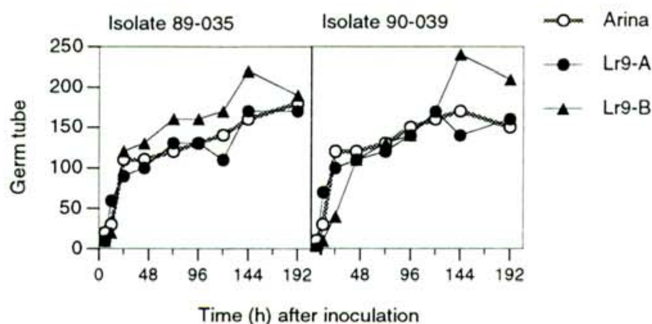


**Fig. 2.** The number of branches produced by germ tubes of 100 spores from *Puccinia recondita* f. sp. *tritici* isolates 89-035 and 90-039 on primary leaves of the susceptible cultivar Arina and its resistant near-isogenic lines (*Lr9*-A and *Lr9*-B).

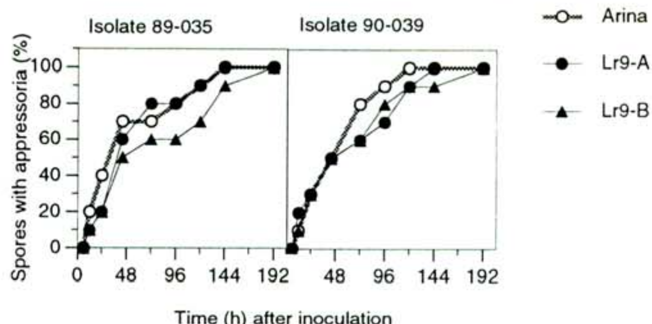


**Fig. 3.** The percentage of spores producing appressoria of *Puccinia recondita* f. sp. *tritici* isolates 89-035 and 90-039 on primary leaves of the susceptible cultivar Arina and its resistant near-isogenic lines (*Lr9*-A and *Lr9*-B).

**Fig. 4.** Development of *Puccinia recondita* f. sp. *tritici* on primary leaves of the susceptible wheat cultivar Arina (**A and B**) and of its leaf rust–resistant, near-isogenic line *Lr9*-A (**C and D**). **(A)** The intercellular hypha, shown here 8 days after inoculation, expanded and colonized the host with hundreds of haustorial mother cells (small fluorescent points), ensuring fungal nutrition. **(B)** The uredinia erupt through the leaf epidermis, producing the macroscopic signs and symptoms of the disease. **(C)** On the resistant host, intercellular growth is inhibited by the necrosis of mesophyll cells (yellow-orange autofluorescence) (36 h after inoculation). **(D)** Necrosis of mesophyll cells normally progresses parallel to the leaf venation, reaching an area of about 10,000 μm$^2$. Ap = appressorium, GT = germ tube, and NT = necrotic tissue.

ror DM 400, and a filter BA 420 were used in combination with the main system. Photography was carried out on Kodak 400 ISO daylight film. The following phases were observed: germination of spores, branching of germ tubes, formation of appressoria, and formation of haustorial mother cells at each infection site. The area of the necrotic autofluorescing sites was measured by means of a hemacytometer (Thoma chamber).

## RESULTS

**Spore germination and appressorium formation.** Urediniospores from both isolates germinated 6 h after inoculation. The germ tubes of all genotypes grew perpendicularly to the venation of the leaf. As soon as the germ tube reached a stoma, growth stopped and an appressorium formed (Fig. 1A and B). Fungal structures appeared more fluorescent than host cells. Fungal structures performing greater metabolic activity were even more fluorescent. Germ tubes appeared as fluorescing strings on the leaf surface and appressoria as enlarged, strongly fluorescing points at their ends. Within the first 24 h after inoculation, most of the spores had germinated (Fig. 2). Close to each leaf vein, short lateral arms of the germ tube were produced (Fig. 1A). Branching of germ tubes was rare during the first 44 h after inoculation (Fig. 2). Afterwards, germ tubes branched more frequently on leaves of the *Lr9*-B line.

Appressorium formation started 6 to 12 h after inoculation (Fig. 3). Within 44 h after inoculation, 50 to 65% of the spores monitored had formed an appressorium. Four days after inoculation, close to 100% of the spores had germinated and had formed an appressorium. No clear effect of *Lr9* on appressorium formation was detected.

**Development of the haustorial mother cell.** Soon after the maturation of the appressorium, the fungus penetrated the stoma with a penetration peg. Romig and Caldwell reported penetration takes place whether stomata are open or closed (17). After the formation of a vesicle in the substomatal cavity, the parasite began to invade the intercellular space of the leaf tissue by a primary hypha. After a single cellular division, the haustorial mother cell was produced. It could be easily recognized in the intercellular spaces of the leaf tissue as a strongly fluorescing cell clearly separated by a septum from the primary hypha (Fig. 1C and D). From the haustorial mother cell, a haustorium was produced in the cell lumen of mesophyll cells. The first haustorial mother cells were detected on Arina between 24 and 44 h after inoculation. On Arina, the number of haustorial mother cells increased exponentially (Figs. 4A and 5). By 196 h after inoculation, 110 haustorial mother cells per colony were recorded for isolate 89-035. Isolate 90-039 had a faster increase of haustorial mother cells, up to 140 haustorial mother cells per colony by 196 h after inoculation. No resistance response by the host inhibited fungal growth, and no necrosis was observed around the infection sites. The first uredinia ruptured the leaf epidermis 7 to 8 days after infection (Fig. 4B).

On resistant lines, a yellow fluorescing material was produced around the infected stomata 24 to 28 h after inoculation (Fig. 4C). This fluorescence, noticed exclusively in association with fungal structures, has been correlated with necrosis of the infected host cells (16). Ninety-six hours after inoculation, the necrotic area around an infection site reached an average area of 10,000 $\mu m^2$ on resistant lines (Fig. 4D). Simultaneously, macroscopically visible leaf spots appeared at the leaf surface. These characteristics are typical for a hypersensitive reaction of a plant against a parasite (4). Both isolates generated only one to three haustorial mother cells before ceasing their growth on the two NILs carrying *Lr9* (Fig. 5).

## DISCUSSION

The parasite was able to penetrate stomata of primary leaves of both susceptible and resistant hosts. On Arina, the number of haustorial mother cells per infection site increased exponentially. However, on both *Lr9* NILs, necrosis of plant tissue took place around the infection sites 24 to 44 h after inoculation. Before ceasing its growth, the fungus generated only a few haustorial mother cells. This result shows that *Lr9* reduced the growth of the leaf rust fungus very early and that the reproductive phase was completely inhibited.

Directional growth of germ tubes and the differentiation of appressoria were similar on both susceptible and resistant hosts. In rust fungi, these developmental processes are regulated by both topographical and chemical features of the leaf surface (8). The perception of such characteristics allows rust fungi to grow perpendicularly to the venation of the leaf (thigmotropic reaction), thus increasing the likelihood of encountering a stoma. Barriers of 0.25 to 1 μm in height spaced 0.5 to 6.7 μm apart represent the optimal physical conditions for a directional growth of the fungal germ tube (9). Appressorium differentiation by *P. recondita* requires barriers with an optimal height of 0.4 to 0.8 μm (1). Rubiales and Niks (18) associated leaf rust resistance of *Hordeum chilense* with the inability of the fungus to locate stomata by means of thigmotropic stimuli. It was suggested that this effect might be due to the presence of wax covering the stomatal opening (19). Such a morphological resistance might affect stomatal conductance and thereby alter the gas exchange rate of the plant. Successful orientation of germ tubes and appressorium differentiation on the resistant hosts were observed in the present work, confirming the observations by Southerton and Deverall (22) and by Jacobs (10), according to whom appressorium differentiation is not affected by race-specific resistance genes.

The necrosis of mesophyll cells as a response of *Lr9* lines to the fungus was only observed after the generation of the first haustorial mother cells. According to Hart (7), stomata of stem rust–resistant wheat cultivars exhibit a more reduced opening interval when compared with susceptible ones, opening later in the morning and closing earlier in the afternoon. It was suggested that stomatal exclusion might represent an effective resistance mechanism against *P. graminis*. This mechanism, classified as functional resistance, might have a direct influence on stomatal conductance and therefore negative effects on plant yield potential. The leaf rust fungus is able to penetrate closed stomata and invade the intercellular spaces of the host tissue, whereas the stem rust fungus lacks this ability (22). This peculiar feature of the leaf rust fungus might be correlated with its ability to grow under very high concentrations of $CO_2$ (26).

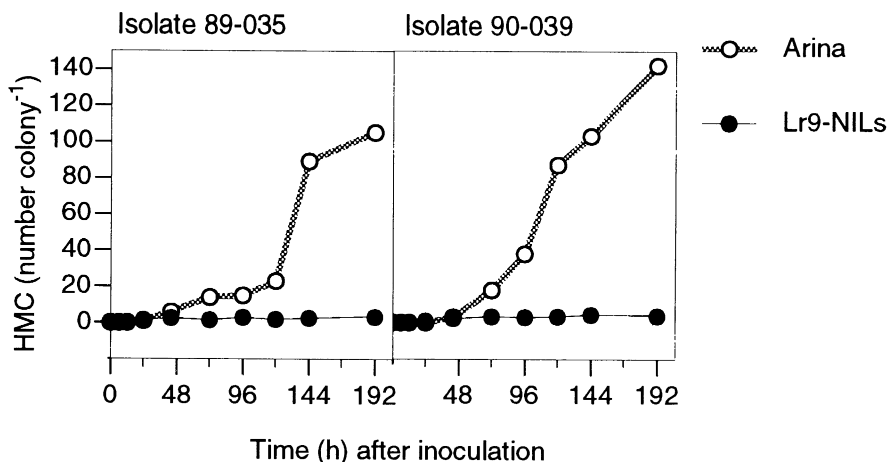


**Fig. 5.** The number of haustorial mother cells (HMC) per colony produced by spores of *Puccinia recondita* f. sp. *tritici* isolates 89-035 and 90-039 on primary leaves of the susceptible cultivar Arina and its resistant near-isogenic lines (*Lr9*-NILs means average of *Lr9*-A and *Lr9*-B).

Cells of the resistant host around the haustorial mother cells showed a hypersensitive reaction and died 24 to 44 h after inoculation. Tiburzy et al. (24) demonstrated that the time lag between haustorium formation and plant response for *P. graminis* was 4.6 to 8.1 h. However, whether tissue necrosis is the cause or the consequence of resistance is not yet established. In fact, the biochemical pathways of the necrosis process remain unclear. After inoculation with an avirulent race of the pathogen, enzyme activities of phenylpropane metabolism increase significantly, particularly in the case of phenylalanine-ammonia-lyase (PAL) and 4-coumarate: CoA-ligase (4CL) (6,14). In the cell walls surrounding the infection site, depositions of callose and products of phenylpropane metabolism, such as phenolic compounds and lignin-like components, were observed (23). By means of in situ mRNA hybridization, the activation of PAL and 4CL genes in regions surrounding necrotic cells could be demonstrated (3). The same authors suggested that the fungus attempts to colonize healthy tissue once it has succeeded in conquering the necrotic area. However, fungal expansion is inhibited by the increased concentration of enzymes and their metabolic products mentioned above. Tiburzy and Reisener (25) and Carver et al. (2) succeeded in increasing susceptibility of cereals to avirulent races of both *P. graminis* and *Erysiphe graminis* by using PAL inhibitors. This evidence suggests that phenylpropane metabolism plays a central role as a host resistance mechanism.

This study shows that *Lr9* is neither a morphological nor a functional type of resistance. The possibility can therefore be excluded that the lower yield of the resistant NILs as compared to Arina observed in earlier experiments (15; *unpublished data*) is directly related to an altered topography of the leaves or reduced stomatal aperture that could increase the boundary layer or stomatal resistance for $CO_2$ and thus reduce photosynthesis. It is more probable that the observed hypersensitive reactions contribute to the lower yield of the resistant NILs because they use energy and assimilates that are no longer available for development of yield. Smedegaard-Petersen and Stølen (21) showed that the increased requirement of energy and structural components leads to a temporary increase in respiration of about 80% in barley infected by an avirulent race of the powdery mildew fungus. Multiple infections with avirulent mildew races and saprophytic fungi resulted in a reduction of the grain yield of 7 to 9% compared to the control (20). In previous field experiments with Arina and the resistant NILs (15), leaf rust infections were observed on Arina in the fall and in early spring. It is therefore possible that hypersensitive reactions on the resistant NILs may already be induced in early stages of development and may require assimilates over a long period of growth. Even a small reduction of assimilates available for growth processes may therefore have an influence on yield formation.

In addition to the use of energy and assimilates, the hypersensitive reaction also reduces the production of assimilates due to the reduction of the photosynthetically active leaf area (Fig. 4C and D). In an inoculation experiment with an avirulent leaf rust race, Southerton and Deverall (22) observed 14% of infected stomata associated with an average necrotic area of 10,230 $\mu m^2$. Assuming a density of 3,000 stomata per $cm^2$, the overall necrotic area should account for 4 to 5% of the leaf surface. Thus, a combination of increased respiration and reduced photosynthetic rate within the necrotic areas may contribute to the reduced yield potential of resistant NILs.

## LITERATURE CITED


1. Allen, E. A., Hazen, B. E., Hoch, H. C., Kwon, Y., Leinhos, G. M. E., Staples, R. C., Stumpf, M. A., and Terhune, B. T. 1991. Appressorium formation in response to topographical signals by 27 rust species. Phytopathology 81:323-331.
2. Carver, T. L. W., Robbins, M. P., and Zeyen, R. J. 1991. Effects of two PAL inhibitors on the susceptibility and localized autofluorescent host cell responses of oat leaves attacked by *Erysiphe graminis* DC. Physiol. Mol. Plant Pathol. 39:269-287.
3. Cuypers, B., Schmelzer, E., and Hahlbrock, K. 1988. In situ localization of rapidly accumulated phenylalanine ammonia-lyase mRNA around penetration sites of *Phytophthora infestans* in potato leaves. Mol. Plant-Microbe Interact. 1:157-160.
4. Freytag, S., and Hahlbrock, K. 1992. Abwehrreaktionen von Pflanzen gegen Pilzbefall. Biol. Unserer Zeit 22:135-142.
5. Fried, P. M., Streckeisen, P., Winzeler, H., Winzeler, M., Forrer, H. R., and Saurer, W. 1992. Development of a multiline for cereal rusts and powdery mildew resistance. Proc. Eur. Mediterr. Cereal Rusts Mildews Conf., 8th. Weihenstephan, Germany. Vortr. Pflanzenzücht. 24:295-298.
6. Fritzemeier, K. H., Cretin, C., Kombrink, E., Rohwer, F., Taylor, J., Scheel, D., and Hahlbrock, K. 1987. Transient induction of phenylalanine ammonia-lyase and 4-coumarate: CoA ligase mRNAs in potato leaves infected with virulent or avirulent races of *Phytophthora infestans*. Plant Physiol. 85:34-41.
7. Hart, H. 1929. Relation of stomatal behavior to stem-rust resistance in wheat. J. Agric. Res. 39:929-948.
8. Hoch, H. C., and Staples, R. C. 1987. Structural and chemical changes among the rust fungi during appressorium development. Annu. Rev. Phytopathol. 25:231-247.
9. Hoch, H. C., Staples, R. C., Whitehead, B., Comeau, J., and Wolf, E. D. 1987. Signaling for growth orientation and cell differentiation by surface topography in *Uromyces*. Science 235:1659-1662.
10. Jacobs, T. H. 1989. Germination and appressorium formation of wheat leaf rust on susceptible, partially resistant and resistant wheat seedlings and on seedlings of other Gramineae. Neth. J. Plant Pathol. 95:65-71.
11. Kramer, C. L., and Eversmeyer, M. G. 1992. Effect of temperature on germination and germ-tube development of *Puccinia recondita* and *P. graminis* urediniospores. Mycol. Res. 96:689-693.
12. Kuck, K. H., Tiburzy, R., Hänssler, G., and Reisener, H. J. 1981. Visualization of rust haustoria in wheat leaves by using fluorochromes. Physiol. Plant Pathol. 19:439-441.
13. McIntosh, R. A. 1988. Catalogue of gene symbols for wheat. Proc. Int. Wheat Genet. Symp., 7th. Cambridge, England. T. E. Miller and R. M. D. Koebner, eds. 2:1225-1323.
14. Moerschbacher, B. M., Noll, U. M., Flott, B. E., and Reisener, H. J. 1988. Lignin biosynthetic enzymes in stem rust infected, resistant and susceptible near-isogenic wheat lines. Physiol. Mol. Plant Pathol. 33:33-46.
15. Ortelli, S., Winzeler, M., Winzeler, H., Fried, P. M., and Nösberger, J. 1992. Reduzierter Körnerertrag bei braunrostresistenten isogenen Winterweizen-Linien: Ertragsphysiologische Grundlagen. Mitt. Ges. Pflanzenbauwissenschaften 5:155-158.
16. Rohringer, R., Kim, W. K., Samborski, D. J., and Howes, N. K. 1977. Calcofluor: An optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology 67:808-810.
17. Romig, R. W., and Caldwell, R. M. 1964. Stomatal exclusion of *Puccinia recondita* by wheat peduncles and sheaths. Phytopathology 54:214-218.
18. Rubiales, D., and Niks, R. E. 1992. Low appressorium formation by rust fungi on *Hordeum chilense* lines. Phytopathology 82:1007-1012.
19. Rubiales, D., Ramirez, M. C., and Niks, R. E. 1992. Poor stoma recognition by rust fungi on some *Hordeum chilense* genotypes. Proc. Eur. Mediterr. Cereal Rusts Mildews Conf., 8th. Weihenstephan, Germany. Vortr. Pflanzenzücht. 24:26-27.
20. Smedegaard-Petersen, V. 1989. Energy costs of plant response to infection. In: Plant-Microbe Interactions. Molecular and Genetic Perspectives. T. Kosuge and E. W. Nester, eds. 3:182-197.
21. Smedegaard-Petersen, V., and Stølen, O. 1981. Effect of energy-requiring defense reactions on yield and grain quality in a powdery mildew-resistant barley cultivar. Phytopathology 71:396-399.
22. Southerton, S. G., and Deverall, B. J. 1989. Histological studies of the expression of the *Lr9*, *Lr20* and *Lr28* alleles for resistance to leaf rust in wheat. Plant Pathol. 38:190-199.
23. Southerton, S. G., and Deverall, B. J. 1990. Histochemical and chemical evidence for lignin accumulation during the expression of resistance to leaf rust fungi in wheat. Physiol. Mol. Plant Pathol. 36:483-494.
24. Tiburzy, R., Noll, U., and Reisener, H. J. 1990. Resistance of wheat to *Puccinia graminis* f. sp. *tritici*: Histological investigation of resistance caused by the *Sr5* gene. Physiol. Mol. Plant Pathol. 36:95-108.
25. Tiburzy, R., and Reisener, H. J. 1990. Resistance of wheat to *Puccinia graminis* f. sp. *tritici*: Association of the hypersensitive reaction with the cellular accumulation of lignin-like material and callose. Physiol. Mol. Plant Pathol. 36:109-120.
26. Yirgou, D., and Caldwell, R. M. 1968. Stomatal penetration of wheat seedlings by stem and leaf rusts in relation to effects of carbon dioxide, light, and stomatal aperture. Phytopathology 58:500-507.