# An Elicitor of the Hypersensitive Lignification Response in Wheat Leaves Isolated from the Rust Fungus *Puccinia graminis* f. sp. *tritici*
# I. Partial Purification and Characterization

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*Triticum aestivum, Puccinia graminis* f. sp. *tritici,* Hypersensitive Response, Lignification, Elicitor

Several biotic and abiotic elicitors of the hypersensitive reaction in wheat leaves are described. The elicitors induce enhanced activity of phenylalanine ammonia-lyase with subsequent lignification, visible as a yellow autofluorescence. The deposited material stains positively with phloroglucinol. DEAE-dextran, epoxystearic acid and chitosan are strong elicitors, while the glucans tested have no activity.

A biotic elicitor (genuine *Pgt*-elicitor) was isolated from the germ tube walls of uredospores of *Puccinia graminis* f. sp. *tritici.* The high molecular weight, water soluble elicitor is heat stable and unaffected by mild acid and mild alkaline treatments. It seems to be a glycoprotein with the carbohydrate moiety bearing the active residues, as indicated by periodate sensitivity and protease stability. The carbohydrate moiety consists mainly of glucose with some galactose and mannose.

Upon ultrafiltration and gel chromatography elicitor activity was associated with fractions of molecular weight of more than 100 kD.

When injected into the intercellular space of primary wheat leaves, the elicitor induces lignification, preceded by an increase in phenylalanine ammonia-lyase activity.

## Introduction

Initially the term "elicitor" was defined as a pathogen metabolite that causes phytoalexin accumulation in plants [1]. However, synthesis of phytoalexins is only one possible resistance response, others being tissue browning, callose accumulation, lignification or electrolyte leakage, *e.g.* Thus the term "elicitor" has since been applied to molecules triggering any resistance response [2]. Elicitors of biological origin are termed biotic, in contrast to abiotic elicitors, which are of physical or chemical nature. However, this division may be artificial and is independent of an actual role in a given host-parasite-interaction [3]. Thus, we prefer the term "genuine elicitor" to describe those biotic elicitors, which 1) are derived from the pathogen, 2) are capable of inducing the appropriate resistance response in the host, and 3) possibly play a role in the natural interaction between host and parasite.

Whereas no phytoalexin has been described for the wheat-stem rust system, lignification in response to fungal attack has been proposed to play an important role in the expression of resistance [4–6]. Recently, Tiburzy [7] described the role of lignification in various incompatible combinations of near isogenic wheat lines and *Puccinia graminis* PERS. f. sp. *tritici* ERICS. & E. HENN. Extreme resistance, as conditioned by the resistance gene *Sr5,* is expressed by hypersensitive cell death. Lignification of the first penetrated cells is proposed to be the decisive resistance mechanism. The first detectable event is a yellow autofluorescent halo round the penetrating haustorium, followed by fluorescence of the whole cell, which is due to the formation of lignin or lignin-like substances. This suggests a host-parasite recognition between a fungal "elicitor" and a host's "receptor" at the haustorium-plasmalemma interface [2].

As shown by Pearce and Ride [8, 9] the lignification response of wheat leaves can be elicited by biotic elicitors.

The aim of this study was to isolate a biotic elicitor from germinated uredospores of *Puccinia graminis* f.

*Abbreviations:* DEAE, diethylaminoethyl; PAL, phenylalanine ammonia-lyase; *Pgt*-elicitor, elicitor-fraction, isolated from germinated uredospores of *Puccinia graminis* f. sp. *tritici; Pmg, Phytophthora megasperma* f. sp. *glycinea;* TFA, trifluoroacetic acid.

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sp. *tritici,* which causes the lignification response in wheat leaves when injected into the intercellular space.

The quantitation of newly synthesized lignin is difficult and time consuming and hence not suitable in a standard assay. Thus, lignification was determined qualitatively by the occurrence of the yellow autofluorescence [7] and the positive phloroglucinol reaction [10].

Induction of phenylalanine ammonia-lyase (PAL) is a common reaction of plants treated with elicitors that stimulate the accumulation of phytoalexins in a wide range of host-parasite interactions [3]. Thus, we also investigated whether elicitors of the lignification response stimulate an increase in PAL activity. This enzyme is proposed to play a regulatory role in the biosynthesis of phenolics, including lignin [11]. PAL activity rises in wheat leaves after inoculation with *Puccinia graminis* f. sp. *tritici* [12]. The same effect is described for inoculation with *Erysiphe graminis* f. sp. *tritici* [5] or with the non-pathogenic *Botrytis* [13].

In this study we report on several biotic and abiotic elicitors of the lignification response in wheat. Furthermore, we describe the partial purification and characterization of wall compounds from *Puccinia graminis* f. sp. *tritici* race 32, causing hypersensitive cell death in leaves of near isogenic wheat lines carrying the *Sr5*-gene for resistance or the corresponding *sr5*-allele for susceptibility.

A preliminary report of some of the presented results has been published previously [14].

## Materials and Methods

### *Plant, fungus and cultivation conditions*

Near isogenic lines of the wheat cultivars Prelude and Marquis carrying the *Sr5*- or *sr5*-allele were received from Dr. Rohringer, Agriculture Canada Research Station, Winnipeg.

Reculturing of the fungus *Puccinia graminis* f. sp. *tritici* race 32, growing and inoculation of the plants were performed as described previously [15]. 7-day-old seedlings of approximately equal size were used in all experiments.

### *Preparation of the glucan elicitor from Pmg*

The glucan elicitor provided by R. Tiemann was prepared by enzymatic digestion of *Pmg* cell walls and shown to induce phytoalexin synthesis on wounded soybean cotyledons (paper in preparation).

### *Preparation of an elicitor active fraction from germinating uredospores*

200 mg of uredospores were floated on 300 ml of water in a petri dish (∅ 18 cm) and allowed to germinate for 20 h in the dark at 20 °C. The following steps were carried out at 4 °C.

Germ tubes were harvested by decanting the water, then washed with an excess of distilled water, ground in a cold mortar, homogenized with a Braun-Melsungen potter and then centrifuged at $1300 \times g$ for 20 min. The turbid supernatant was centrifuged at $65000 \times g$ for 30 min. The resulting pellet was washed with 10 ml of a chloroform/methanol mixture (2/1 v/v) and lyophilized overnight. This crude elicitor preparation was stored at −19 °C or worked up immediately. The powder was resuspended in 300 µl of distilled water, extensively homogenized in a potter and clarified by a subsequent centrifugation at $65000 \times g$ for 30 min. The resulting clear solution contains the water soluble biotic elicitor. After estimation of the carbohydrate and protein content, the preparation was lyophilized overnight.

In the following, the term "*Pgt*-elicitor" refers to this elicitor active fraction.

### *Assay for elicitor activity*

The solutions to be tested for elicitor activity were injected into the intercellular space of 7-day-old primary leaves as described previously [15], using a hypodermic syringe.

### *Assay for lignification response*

40 h after injection the leaves were harvested, fixed in 80% ethanol, heated in boiling water for 3 min, cooled down, washed with water and transferred to glycerol. Autofluorescence of lignin or lignin-like substances was observed with a Zeiss epifluorescent photomicroscope using a filter combination G 365, FT 395, LP 420 according to Tiburzy [7].

The phloroglucinol stain for lignin was applied as described by Sherwood and Vance [10].

### *Assay for induced PAL activity*

24 h after injection 4 primary leaves were harvested and identical sections (3 cm from the injection

site to each side) were immediately frozen in liquid nitrogen.

PAL activity was determined using a modified procedure of Green *et al.* [5]. The frozen leaf sections were ground in a cold mortar containing 4 ml of borate buffer (0.1 M, pH 8.8) and an abrasive. The homogenate was transferred to a centrifuge tube, mortar and pestle rinsed twice with 1 ml of borate buffer. The supernatant of the subsequent centrifugation at $20000 \times g$ for 15 min was used as enzyme extract. 300 µl of this extract were incubated with 0.6 ml of borate buffer containing 6 µmol of L-phenylalanine. The reaction velocity was shown to be linear with time for at least 3 h. After 2 h the reaction was stopped by addition of 0.1 ml of 6 N HCl. The cinnamic acid formed was extracted by adding 1 ml of chloroform, vigorous shaking and centrifuging for 5 min at $1300 \times g$. A 0.5 ml aliquot of the chloroform phase was taken, the chloroform allowed to evaporate. The residue was resuspended in 0.5 ml of borate buffer. The extinction was measured in a spectrophotometer at 270 nm. Cinnamic acid (0.1–10 µg/ml) served as a standard. Enzyme activity was calculated in pkat/g fresh weight.

*Enzymatic degradations*

400 µg (glucose equivalents) of the elicitor were dissolved in 1 ml of the appropriate buffer for all enzyme digestions.

Chitinase digestion was carried out in acetate buffer (80 mM, pH 5.0) containing 20 µg of chitinase (12 units/mg, Sigma). After 24, 48 and 72 h of incubation at 37 °C aliquots were taken, and 15 µg of new enzyme was added to the reaction mixture. N-acetylglucosamine content of the aliquots was determined as described below. Controls contained heat inactivated chitinase. Crab chitin (Sigma, Roth) was used to check enzyme activity.

Pronase digestion of the elicitor was carried out in Tris-HCl buffer (100 mM, pH 7.9) containing 250 µg of pronase (45 PUK/mg, Calbiochem) and 2 µmol $CaCl_2$. Papain and laminarinase digestions were carried out in ammonia acetate buffer (50 mM, pH 5.0) containing 50 µg of papain (24 units/mg, Sigma), 5 µmol cysteine and 2 µmol EDTA, or 1 µg of laminarinase (25 units/mg, Sigma). Controls contained heat inactivated pronase, papain or laminarinase. BSA (Serva) or laminarin (Calbiochem) were used to check enzyme activities. Incubation was 24 h at 37 °C.

*Acid and alkaline treatment*

200 µg (glucose equivalents) of the elicitor were dissolved in 1 ml of 0.5 N KOH or 0.1 N TFA and incubated for 1 h at 100 °C (mild conditions). Acid hydrolysis was carried out with 0.5 N TFA (2 h, 100 °C). After incubation the samples were neutralized with 1 N HCl or 1 N NaOH and dialyzed twice overnight against 500 ml of distilled water.

*Periodate oxidation*

Periodate oxidation was carried out according to a modified procedure of de Wit and Roseboom [16].

200 µg of elicitor (glucose equivalents) were incubated with 1 ml of $NaJO_4$ solution (0.05 M) for 24 h at 4 °C in the dark. Excess $JO_4^-$ was reduced by addition of 0.1 ml of ethylene glycol. In a control experiment 0.1 ml of ethylene glycol were incubated with 1 ml of $NaJO_4$ solution (0.05 M) for 2 h at 4 °C prior to addition of elicitor. Samples were dialyzed twice overnight against 500 ml of distilled water.

*Biochemical analyses*

Protein content was determined by the methods of Lowry *et al.* [17] and Gornall [18], carbohydrate content with the anthrone reagent by the method of Morris [19].

Analysis of amino acid composition was kindly carried out by Dr. Föhles from Deutsches Wollforschungsinstitut, Aachen, using a physiological program on a Biotronic LC 5000 automatic analyzer.

Aminosugar content was kindly determined by Dr. Stuhlsatz from the Abteilung für Klinische Chemie of the RWTH Aachen on a Biotronic LC 4000/6000 automatic analyzer with a modified program of hydrolysate amino acids, that allows simultaneous determination of hexosamines and amino acids.

To determine the monosaccharide composition gas-liquid chromatographic analyses were carried out according to Jones and Albersheim [20], injecting the samples in acetonic solution. Column and run specifications were as follows: 0.2% of (poly)ethylene glycol succinate, 0.2% of (poly)ethylene glycol adipate and 0.4% of GE-silicone × F 1150 coated on gaschromosorb P 100–200 mesh (applied science) in a 1.2 m by ⅛ inch outside diameter stainless steal column; temperature program: 5 min post injection hold at 170 °C, followed by a linear 1 °C/min temperature rise to 195 °C, hold at 195 °C for 15 min. A nitrogen carrier gas flow of 60 ml/min was main-

tained. The injection port temperature and the dual hydrogen flame ionization detector temperature were 250 °C.

Glucosamine content was determined using the method of Ride and Drysdale [21]. N-acetylglucosamine was determined by the method of Reissig *et al.* [22]. Reducing sugars were determined using the method of Nelson [23] and Somogyi [24].

## Results

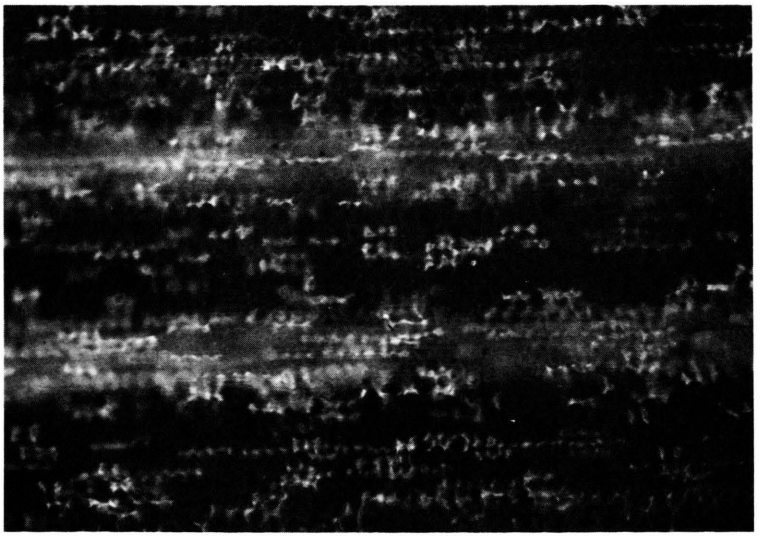
### *Lignification response and induction of PAL activity*

Several abiotic and biotic elicitors of the lignification response in wheat leaves were tested, using the near isogenic wheat line Prelude carrying the *Sr5*-gene for resistance or the corresponding *sr5*-allele for susceptibility (Table I). A 0.1% (w/v) aqueous solution of diethylaminoethyl(DEAE)-dextran has a strong effect preferentially on mesophyll cells. A 0.001% (w/v) aqueous suspension of chitosan is an effective elicitor for epidermal as well as for mesophyll cells. In these cases the lignified cells form a regular pattern over the whole area soaked with the test solutions (Fig. 1). A 0.01% (w/v) aqueous suspension of ethanol solubilized epoxystearic acid induces strong lignification restricted to an area round the injection site, with no effect on the more distant leaf sections (Fig. 2).

In contrast the *Pgt*-elicitor shows its greatest effect on the border of the soaked area, where epidermal as well as mesophyll cells lignify, whereas in the area between injection site and border of the soaked area only few mesophyll and epidermal cells are affected (Fig. 3).

Table I. Elicitor activity of some abiotic and biotic compounds, injected into the intercellular space of 7-day-old primary leaves of the near-isogenic wheat line Prelude carrying the *Sr5*- or *sr5*-allele. Lignification was detected 40 h after injection by its yellow autofluorescence (see Figures 1 to 3). PAL activity was determined 24 h after injection. Injection of water alone did not lead to an increase in PAL activity beyond the level of untreated controls. Only the *Pgt*-elicitor induced significantly higher levels of PAL activity in resistant plants compared to susceptible plants (t-test, $p = 0.05$). Data given represent the average values of at least 3 independent experiments. The differential PAL inducing capacity of the *Pgt*-elicitor was obtained in more than 10 different preparations.

| Test solution | Lignification | | PAL activity pkat/g f. wt | |
|---|---|---|---|---|
| | *Sr5* | *sr5* | *Sr5* | *sr5* |
| 0.001% chitosan | ++ | ++ | 311 | 285 |
| 0.1% chitin | – | – | 41 | 24 |
| 0.01% epoxystearic acid | ++ | ++ | 14 | 18 |
| 0.1% DEAE-dextran | ++ | ++ | 397 | 425 |
| 0.5% laminarin | – | – | 35 | 32 |
| 0.5% lichenin | – | – | 30 | 33 |
| 0.5% pustulan | – | – | 25 | 12 |
| 0.5% pullulan | – | – | 51 | 7 |
| 0.05% glucan elicitor from *Pmg* | – | – | 46 | 58 |
| 0.04% *Pgt*-elicitor | + | + | 1052 | 628 |
| 0.0001% *Pgt*-elicitor | – | – | 150 | 96 |
| distilled water | – | – | 26 | 25 |
| untreated leaves | – | – | 36 | 23 |



Figs 1 to 3. Lignification response of 7-day-old primary leaves of the near-isogenic wheat line Prelude carrying the resistance gene *Sr5*, 40 h after injection of elicitors into the intercellular space, as seen in the epifluorescence microscope.

Fig. 1. After injection of DEAE-dextran (0.1%), lignified cells form a regular pattern over the whole soaked area.

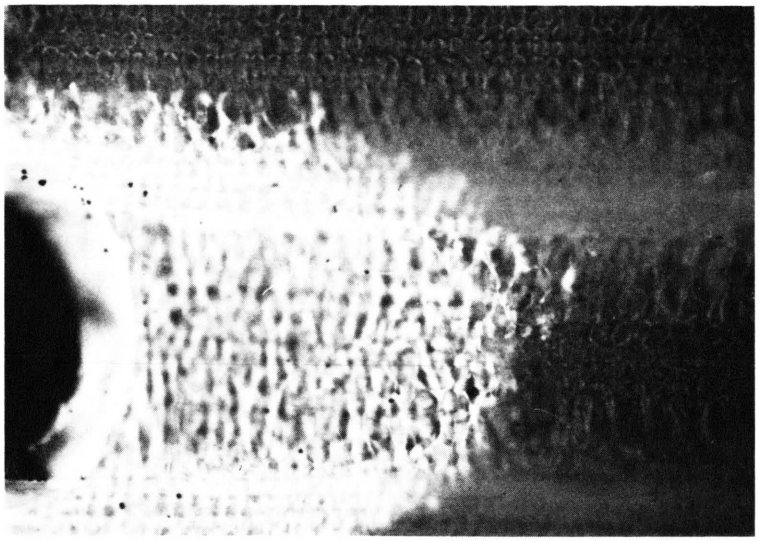


Fig. 2. After injection of expoxystearic acid (0.01%), lignification is restricted to an area round the injection site.

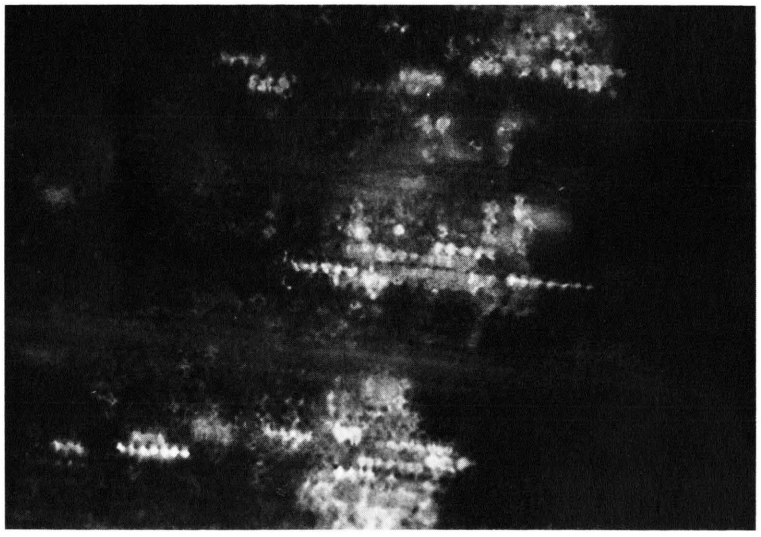


Fig. 3. In contrast to Figures 1 and 2, the genuine biotic elicitor from *Puccinia graminis* f. sp. *tritici* (0.04% glucose equivalents) leads to a strong lignification on the border of the soaked area (middle of the plate) with only few lignified cells between injection site and border of the soaked area (left side of the plate). This observation may point to the presence of a diffusible factor that is washed out of the soaked area during injection of the elicitor, and which is necessary to mediate the elicitor activity.

Several glucans were tested for elicitor activity (0.5% (w/v) aqueous solutions). Neither the β-glucans laminarin (β-1.3.), lichenin (β-1.4.), pustulan (β-1.6.) and the glucan elicitor from *Phytophthora megasperma* f. sp. *glycinea* (β-1.3., β-1.6.), nor the α-glucan pullulan (α-1.6.) induce lignification.

Upon treatment with any of the above mentioned effective elicitors lignification occurred in both the susceptible and the resistant line. However, the microscopic observations do not allow the quantitation of the lignin present. In order to quantify the effects of the different elicitors with respect to the presence or absence of the *Sr5*-gene for resistance, we determined the increase in PAL activity of wheat leaves after injection of the elicitor preparations. Elevated PAL activity always preceded the lignification response, the only exception being epoxystearic acid (Table I). The highest activities ever observed were reached after injection of the *Pgt*-elicitor.

The *Pgt*-elicitor retains activity at a concentration as low as 1 μg/ml, which is approximately 30 ng per injected leaf.

In contrast to the other elicitors the *Pgt*-elicitor stimulates PAL significantly more strongly in resistant than in susceptible plants.

These results were confirmed in experiments with the near isogenic wheat line Marquis, carrying the *Sr5*- or *sr5*-allele and with the cultivars Feldkrone (highly resistant, probably due to the *Sr5*-gene [7]) and Little Club (susceptible).

### *Characterization of the Pgt-elicitor*

The germ tube elicitor was prepared as described under Materials and Methods. A typical preparation contains approximately 1.50 mg polysaccharide (glucose equivalents) and 0.75 mg protein (BSA equivalents) per gram of uredospores used.

Gas-liquid chromatographic analysis shows that the polysaccharide moiety consists mainly of glucose (90%) with some mannose (4%) and galactose (6%), whereas only traces of glucosamine and/or N-acetylglucosamine are present.

Glucosamine is found at very low concentrations in the colorimetric assay (approximately 1% of total carbohydrate).

Treatment with chitinase does not liberate N-acetylglucosamine and has no effect on the elicitor activity. This does not necessarily imply a lack of chitin, because any chitin present might not be accessible to chitinase digestion.

Hexosamine determination on an automatic amino acid analyzer, also revealed only a small amount of glucosamine and/or N-acetylglucosamine (approximately 0.5% of total carbohydrate) and no galactosamine.

Amino acid composition of the protein moiety is shown in Table II. There is one peak closely behind the alanine peak which has not yet been identified. The values obtained from 3 independent preparations indicate the high reproducibility of the purification method.

Enzymatic digestion with laminarinase does not liberate any reducing sugars and has no effect on the elicitor activity, suggesting that the glucan is not β-1.3.-linked. Digestion with either papain or pronase had no influence on elicitor activity (Table III). Periodate, on the other hand, completely destroys the activity, suggesting that the carbohydrate moiety bears the active residues. The elicitor activity is heat stable even after autoclaving for 3 h at 121 °C.

Table II. Amino acid composition of the biotic elicitor preparation from *Puccinia graminis* f. sp. *tritici*. The elicitor was hydrolyzed in 6 N HCl for 24 h at 110 °C. Analyses were carried out on an automatic amino acid analyzer, using a physiological program. There is a yet unidentified peak shortly behind the alanine. The values obtained from 3 independent preparations indicate the high reproducibility of the purification method.

| Amino acid | mol/100 mol aa | | |
|---|---|---|---|
| Asp | 9.817 | 9.374 | 10.217 |
| Hypro | – | – | – |
| Met = O | 0.148 | 0.111 | 0.209 |
| Thr | 8.270 | 7.431 | 8.632 |
| Ser | 10.099 | 9.808 | 10.614 |
| Asn | – | – | – |
| Glu | 10.905 | 10.459 | 10.753 |
| Gln | – | – | – |
| Pro | 6.885 | 6.747 | 6.640 |
| Gly | 8.671 | 7.569 | 8.436 |
| Ala | 10.863 | 10.456 | 11.275 |
| Cit | – | – | – |
| Val | 5.386 | 4.683 | 5.020 |
| (Cys)2 | 0.563 | 0.422 | 0.438 |
| Met | 0.828 | 0.745 | 0.603 |
| Ile | 3.302 | 10.042 | 3.331 |
| Leu | 6.588 | 5.730 | 6.334 |
| Asp-Lys | – | – | – |
| Glu-Lys | – | – | – |
| Tyr | 1.971 | 1.619 | 1.884 |
| Phe | 3.632 | 3.344 | 3.644 |
| HO-Lys | – | – | – |
| Orn | 0.061 | 0.058 | 0.072 |
| Lys-Ala | – | – | – |
| Lys | 6.794 | 6.645 | 6.960 |
| His | 1.926 | 1.825 | 1.905 |
| Trp | – | – | – |
| Arg | 3.292 | 2.932 | 3.032 |
| Lan | – | – | – |
| Me-His | – | – | – |

Acid hydrolysis with 0.5 N TFA completely destroys the activity. Mild acid and mild alkaline treatments did not affect elicitor activity.

As determined by ultrafiltration, the molecular weight of the *Pgt*-elicitor is higher than 100,000. Gel chromatography on Bio-Gel A-1.5 m (Biorad) and sepharose 2B (Pharmacia) columns with distilled water, 100 mM NaCl or 10 mM phosphate buffer pH 7.0 as eluent suggests, that the active compound(s) is a polydisperse material with a molecular weight ranging from 100 up to > 40,000 kdalton, possibly indicating complex formation.

Active fractions always contained carbohydrate and protein.

Table III. Lignification response of 7-day-old primary leaves of the near-isogenic wheat line Prelude carrying the *Sr5*-gene for resistance or the corresponding *sr5*-gene for susceptibility. After the indicated treatment (for details see under Materials and Methods), the genuine elicitor from *Puccinia graminis* f. sp. *tritici* was injected into the intercellular space of the leaves. Lignification was determined 40 h after injection by the occurrence of the yellow autofluorescence of lignin or lignin-like substances.

| Treatment of the *Pgt*-elicitor | Lignification | |
|---|---|---|
| | *Sr5* | *sr5* |
| pronase | + | + |
| heat-inactivated pronase | + | + |
| papain | + | + |
| heat-inactivated papain | + | + |
| laminarinase | + | + |
| heat-inactivated laminarinase | + | + |
| chitinase | + | + |
| heat-inactivated chitinase | + | + |
| periodate | − | − |
| ethyleneglycol treated periodate | + | + |
| 0.5 N KOH (1 h, 100 °C) | + | + |
| 0.1 N TFA (1 h, 100 °C) | + | + |
| 0.5 N TFA (2 h, 100 °C) | − | − |
| heat (3 h, 121 °C) | + | + |

## Discussion

### *Test system for elicitor activity*

Pearce and Ride [8, 9] showed that treatment with biotic elicitors can stimulate the lignification response of wheat leaves. These authors used wounded primary leaves for the application of test solutions, stating that wounding alone did not cause any lignification. In our experiments, mechanical injury of leaves always led to the formation of lignin. Furthermore, Thorpe and Hall [25] reported on increased PAL and peroxidase activities of non-inoculated wounded wheat leaves compared to healthy controls.

Therefore we injected the test solutions into the intercellular space of primary leaves. In control plants injected with water alone, lignification was limited to the injection site, whereas in the soaked area the test solution came into close contact with mesophyll and epidermal cells without any mechanical wounding.

Elicitor activity was judged by the appearance of the yellow autofluorescence due to the formation of lignin or lignin-like substances [7], the positive phloroglucinol reaction [10, 7] and by the increase in PAL activity, one of the key enzymes in the biosynthesis of lignin. Injection of water alone did not affect the PAL activity (see Table I). Appearance of the yellow autofluorescence was always correlated with a preceding increase in PAL activity. The only exception was epoxystearic acid that caused a strong lignification without a detectable increase in PAL activity. Epoxystearic acid was not inhibitory in the *in vitro* PAL assay. We presume that epoxystearic acid acts as a toxic compound that rapidly kills the wheat cells, so that no active enzyme could be extracted from the leaves 24 h after application.

### *Biotic and abiotic elicitors*

None of the tested glucans had any elicitor activity in our experiments, whereas Pearce and Ride reported a weak activity of laminarin [9]. Chitin suspensions had no elicitor activity. This does not necessarily contradict the results of the above authors, who reported activity of chitin suspensions. The different results may be due to the different application methods for test substances. As suggested by these authors themselves wounding may activate chitinase activity, which transforms the insoluble chitin into a more soluble form that has elicitor activity.

The different assay procedures may also account for the fact that chitosan showed only weak activity in the former study, whereas we found it to be an effective elicitor even at concentrations as low as 10 µg/ml. Chitosan is a well known elicitor in several host-pathogen interactions [26, 27]. Hadwiger and Loschke [26] suggested that chitosan may be liberated enzymatically from fungal chitin-glucan complexes. This may provide another explanation for the elicitor activity of chitin in wounded leaves.

Strong lignification was observed after injection of DEAE-dextran (1 mg/ml) as described by Harder *et al.* [28]. This is probably due to plasmamembrane disorganization [29], as also indicated by the fact that we found the poly-cation poly-L-lysine to be an effective elicitor, whereas the poly-anion dextran-sulphate showed no activity (unpublished results of the authors).

Several fatty acids have been reported to act as elicitors of phytoalexin accumulation [30]. Thus the observed effect of epoxystearic acid may be important in view of the fact that epoxystearic acid is a constituent of rust uredospores [31].

### *Genuine Pgt-elicitor*

In the last few years a range of biotic elicitors isolated from plant parasites have been described, that

induce the accumulation of phytoalexins in host and non-host plants [2, 32].

Pearce and Ride [8, 9] reported on biotic elicitors and crude preparations of unidentified genuine elicitors of the lignification response in wheat leaves. There are some earlier studies upon genuine biotic elicitors of necrotization in wheat. Silverman [33] described a "toxin" isolated from rust infected leaves of susceptible wheat plants, that elicits a necrotic reaction in resistant wheat leaves. Rohringer and coworkers reported that RNA preparations function as elicitors of the hypersensitive response [34], but the initially observed specific elicitation of necrosis could not be confirmed in later work [35].

With the exception of race and cultivar specific elicitors from the intercellular fluid from *Cladosporium* infected tomato leaves [36], no elicitor has yet been described that works exclusively in resistant plants and has no effect in susceptible plants. However, in some studies resistant plants react more sensitively than susceptible ones [2], indicating that the susceptible and resistant reaction may differ in quantitative rather than in qualitative aspects. The observed difference in PAL activity after treatment of wheat leaves with the *Pgt*-elicitor may point to a similar specific effect in our system.

The genuine elicitor from germ tube walls of the wheat stem rust is a high molecular weight, water soluble compound, probably a glycoprotein. The elicitor activity is unaffected by pronase and papain digestions, but sensitive to periodate oxidation, indicating that the carbohydrate moiety contains the active part of the molecule(s). However, periodate also oxidizes some amino acids and other compounds such as the aminogroup of chitosan, that might play an important role. Although chitosan is a potent elicitor of the lignification response in wheat leaves, the amount of glucosamine in the preparation of the *Pgt*-elicitor seems too small to account for the measured increase in PAL activity, as seen by comparison with the PAL inducing activity of pure chitosan. Hence, the elicitor activity probably is mediated by the glucan moiety.

Since Pearce and Ride observed a weak activity of laminarin, a predominantely β-1.3.-linked glucan [9], we attempted to digest the *Pgt*-elicitor with laminarinase. The failure of this digestion as well as the sensitivity to periodate indicate, that the glucan is not β-1.3.-linked.

Omitting the chloroform/methanol washing leads to a crude preparation, that has elicitor activity when injected into wheat leaves, showing that elicitor active material can be extracted from the fungal wall by aqueous extraction alone without the use of harsh, unphysiological methods.

An equally active elicitor preparation can be isolated from rust hyphae grown in axenical culture on a medium based on wheat leaf constituents [37]. Although the chemical composition of germ tube walls and walls of axenically grown hyphae differ markedly [38, 39], the elicitor active compound seems to be a common constituent of both.

The germination water of the uredospores also contains elicitor active material, probably due to autolysis of germ tubes, as reported for other biotic elicitors [2].

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