A Water-soluble Extract from Germlings of *Erysiphe pisi* Suppresses Infection by *E. graminis* in Coleoptile Cells of Barley*

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Abstract

Water-soluble, extracellular materials from germlings of *Erysiphe pisi* (a nonpathogen of barley) suppressed infection by *E. graminis* (a pathogen of barley) in barley coleoptiles by about 50%. The suppressive activity was associated with a low molecular weight, heat-stable moiety. The active preparation was separated into three fractions each having suppressive activity. One fraction suppressed infection by *E. graminis* by about 95%. Treatment of coleoptiles with various concentrations of the fractions prior to, or after inoculation with *E. graminis* suggested that at least 6 hr was required before suppression of infection was measurable. The effect was diminished within 10 hr after it reached a level sufficient to suppress the infection by *E. graminis* by about 50%. The results suggested that water-soluble extract of *E. pisi* either enhanced inaccessibility of coleoptile cells (elicitor activity) or directly affected penetration by *E. graminis*.

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Key words: *Erysiphe graminis*, *E. pisi*, elicitor-like factor, inaccessibility.

INTRODUCTION

Components of fungal or host origin, when applied to plants or plant parts, are able frequently to elicit synthesis of the same phytoalexin as is elicited by pathogen attack⁵⁾. The term "elicitor" was originally proposed by Keen⁷⁾ to designate molecules and other stimuli that induced phytoalexin synthesis in plant cells. It is now commonly used to connote molecules and/or stimuli that elicit any plant defense response. To date a variety of cell wall components²³⁾ and intra- or extra-cellular components of fungi^{8,24)} are reported to elicit phytoalexin accumulation and other defense responses in various plants. Although many studies have shown that a variety of obligate parasites induce plant defense reactions like papilla formation²⁾, hypersensitive cell death^{3,21)} and phytoalexin accumulation¹⁷⁾, the demonstration of elicitors from obligate parasites is limited⁶⁾.

Our cytological investigations^{12,13,15)} demonstrated that when the nonpathogen, *Erysiphe pisi*, attempted penetration of a coleoptile cell of barley, the cell was conditioned to reject the pathogen, *E. graminis*. In another report¹⁴⁾, we showed that when *E. pisi* germings were removed from coleoptile cell

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surfaces by micromanipulation at, or 2 hr after, appressorium maturation, the cells were conditioned towards the inaccessible state and suppressed infection by *E. graminis* by about 50%. These results led us to assume that *E. pisi* germlings might release an elicitor(s) during appressorium maturation and that the putative elicitor presumably enhances inaccessibility.

In this study we obtained such substances from *E. pisi* germlings grown on glass plates where initial germling morphogenesis was similar to that occurring on plant surfaces¹⁶⁾.

MATERIALS AND METHODS

Fungi. *Erysiphe pisi* DC. race I was used for preparation of a water-soluble extract, and *E. graminis* DC. f. sp. *hordei* Em Marchal race I as a test fungus for determining the condition of host cells. *E. pisi* was maintained on its host, pea (*Pisum sativum* L., cv. Midoriusui), grown at $20 \pm 2^\circ\text{C}$ and 70% relative humidity (RH) under natural light in a biotron. *E. graminis* was maintained on barley (*Hordeum vulgare* L., cv. Kobinkatagi) grown at $20 \pm 2^\circ\text{C}$ and 70% RH under fluorescent lights (11.8 W/m^2 for 12 hr/day).

Preparation of a water-soluble extract from *E. pisi* germlings. Conidia of *E. pisi* were inoculated onto petri dishes (ca. 18 cm in diameter) with a brush, and incubated at 20°C with 100% RH in the dark. Inoculum density was about 1,500 conidia/mm². When the incidence of appressoria reached a maximum, 9 hr after inoculation¹⁶⁾, 80 ml of distilled water was poured into inoculated petri dishes and shaken gently for 20 min to obtain a water-soluble extract from the germlings. The germling suspension was filtered through glass filters (Whatman, GF) and millipore filters (Millipore Co., pore size: $0.22 \mu\text{m}$), to obtain a germling-free filtrate. The filtrate was lyophilized and stored in a freezer. To collect the lyophilized material, 200 petri dishes were used, and approximately 1 mg of material was obtained from ca. 10^6 conidia. The activity of lyophilized material to suppress the *E. graminis* infection was examined.

Bioassay of the water-soluble extract. Barley cv. Kobinkatagi was grown from seed under fluorescent lights (11.8 W/m^2 , 14 hr/day). Coleoptiles were excised from seedlings 9 days after sowing, and single cell epidermal layers of partially dissected coleoptiles were prepared as described previously²⁰⁾. The partially dissected coleoptiles were floated on 0.01 M CaCl_2 solution which had been shown to enhance the infection of *E. graminis*²⁰⁾ and had been used in the bioassay of accessibility and inaccessibility of coleoptile cells in our past studies¹²⁻¹⁵⁾.

Lyophilized material was dissolved in distilled water at a concentration of 5 mg/ml and centrifuged at $4,500 \times g$ for 10 min. The supernatant was used as the stock solution of water-soluble extract. Extract solutions ranging in concentration from 0.2 to 2 mg/ml were prepared from the stock solution using 0.1 M CaCl_2 . The final concentration of CaCl_2 in extract-preparations was 0.01 M. The lower sides of coleoptiles were treated with $50 \mu\text{l}$ of the respective extract solutions in watch glasses, and upper sides were inoculated with 100-150 freshly harvested conidia of *E. graminis*, and incubated at 20°C . Twenty-four hours after the onset of incubation, penetration efficiency (PE: the percentage of appressoria that formed a haustorium) was determined by light microscopy. For a control, inoculated coleoptiles were treated with 0.01 M CaCl_2 solution without the extract, and PE was determined. The activity of the extract to suppress the *E. graminis* infection was evaluated by comparing PEs in extract-treated and control coleoptiles using Student's *t*-test.

To examine whether suppression of PE was due to enhanced inaccessibility in host cells or to fungitoxicity of the extract preparation, the morphogenesis of *E. graminis* on extract-treated coleoptile cells was evaluated. The incidences of primary germ tubes and appressoria, and the percentage of papilla formation induced by appressoria were determined 24 hr after inoculation.

Pre- and post-treatment of coleoptiles with the extract. The time required for measurable suppression of the *E. graminis* infection in extract-treated coleoptile cells was examined. The lower sides of coleoptiles were first treated with the extract solution of 2 mg/ml for 1-12 hr. After washing treated sides with distilled water several times, the upper sides were inoculated with conidia of *E. graminis*. Coleoptiles were then floated on 0.01 M CaCl_2 in petri dishes for 24 hr and PEs were determined (Fig. 1).

In another experiment, upper surfaces of coleoptiles were first inoculated with *E. graminis*, floated on an extract solution of 2 mg/ml and incubated for given times (Fig. 2). At the indicated times, lower surfaces previously treated with the extract were washed with distilled water, and coleoptiles were transferred onto 0.01 M CaCl₂ solution, followed by further incubation. PEs were determined 24 hr after inoculation as stated above.

Treatment of coleoptiles with fractionated or heated extract. Five mg/ml of the stock extract solution was separated into fractions of lower and higher molecular weights by ultrafiltration (Millipore Co., LGC 24). Lower surfaces of coleoptiles were treated with either fraction for 24 hr after inoculation of upper surfaces with *E. graminis* and PEs were determined.

Five mg/ml of the extract solution was further fractionated by high performance liquid chromatography (HPLC) (Tosoh: column, Tsk-gel PW 3000G). Before HPLC analysis, the solution was centrifuged at $4,500 \times g$ for 10 min to remove debris. The column was eluted with distilled water. The elution profile of the preparation was monitored at 210 nm and is illustrated in Fig. 3. The fractions designated as F1 through F4 were collected with a fraction collector. Each fraction was lyophilized and its dry weight was determined. These fractions were bioassayed for their activity to suppress the *E. graminis* infection.

The stock extract solution (5 mg/ml) was heated in a water bath at 100°C for 30 min. It was then diluted with distilled water and 0.1 M CaCl₂ to a final concentration of 2 mg/ml in 0.01 M CaCl₂. Suppression of the *E. graminis* infection by this heat-treated extract was bioassayed as described above.

RESULTS

Effects of the water-soluble extract on the *E. graminis* infection

As shown in Table 1, PE of *E. graminis* was 81.0% on average in coleoptiles whose lower sides were not treated with the extract (control). When coleoptiles were treated with 2 mg/ml of the extract, PE was significantly ($P < 0.01$) reduced to 46.9%. When coleoptiles were treated with 1, 0.5 and 0.2 mg/ml of

Table 1. Effects of varied concentrations of the *E. pisi* extract on penetration efficiency of *E. graminis* in coleoptile cells (Lower sides of coleoptiles were treated with the extract from the onset to the end of incubation)

Concentration of extract (mg/ml)	Penetration efficiency	Percentage of suppression of infection ^{a)}
2.0	46.9 ± 2.8 ^{b)}	42.1
1.0	67.9 ± 4.6 ^{c)}	16.2
0.5	74.5 ± 3.0 ^{c)}	8.0
0.2	75.1 ± 2.1 ^{c)}	7.3
0 (control)	81.0 ± 1.8	—

a) Percentage of suppression of infection

= [1 - (PE of treated coleoptiles/PE of control coleoptiles)] × 100.

b) Significantly different from PE of control ($P < 0.01$).

c) Significantly different from PE of control ($P < 0.05$).

Table 2. Effects of the *E. pisi* extract on morphogenesis of *E. graminis* on coleoptiles

Treatment with extract	Germination ^{a)}	Percentage of morphogenesis Appressorium ^{b)}	Papilla formation ^{c)}
Yes	87.5 ± 2.8	83.6 ± 2.1	92.1 ± 3.1
No	90.7 ± 1.9	75.5 ± 3.3	85.2 ± 3.6

a) Germination (%)

= (No. of conidia with a primary germ tube/No. of observed conidia) × 100.

b) Appressorium (%)

= (No. of conidia with an appressorium/No. of observed conidia) × 100.

c) Papilla formation (%)

= (No. of appressoria which induced a papilla in a coleoptile cell/No. of conidia with an appressorium) × 100.

the extract, PEs were 67.9, 74.5 and 75.1% on average, respectively. These PEs were also significantly lower than that of control ($P < 0.05$). The percentages of suppression of infection ranged from 7.3 to 42.1% at concentrations of extract between 0.2 and 2.0 mg/ml. *E. graminis* conidia usually produce first a primary germ tube and then an appressorial germ tube¹¹⁾. As indicated in Table 2, morphogenesis of *E. graminis* conidia, from formation of primary germ tubes and appressoria, followed by their penetration occurred similarly on coleoptiles treated or untreated with 2 mg/ml of the extract. Fungal morphogenesis up to penetration attempt was not affected by the extract.

Effects of pre- and post-treatment of coleoptiles with the extract on the E. graminis infection

PEs of inoculated *E. graminis* ranged from 73.6 to 82.7% on coleoptiles pre-treated with 2 mg/ml of the extract for 1-12 hr prior to inoculation (Fig. 1). These PEs were not significantly different from that of control coleoptiles.

On the other hand, the post-treatment of inoculated coleoptiles for various hours after inoculation

Treated with <i>E. pisi</i> extract (----)	Inoculation with <i>E. graminis</i> ↓	Treated with 0.01 M CaCl ₂ (---)	<i>E. graminis</i> PE (%)
----- 12 hr -----			73.6
----- 9 hr -----			79.3
----- 6 hr -----			82.7
----- 3 hr -----			81.3
----- 1 hr -----			82.7
		24 hr	
Control			81.7

Fig. 1. Effects of pre-treatment of coleoptiles with the *E. pisi* water-soluble extract prior to inoculation on penetration efficiency (PE) of *E. graminis*. All the PEs are not significantly different ($P < 0.01$).

Inoculation with <i>E. graminis</i> ↓	Treated with 0.01 M CaCl ₂ (---)	<i>E. graminis</i> PE (%)
- 1 hr -		79.6
----- 3 hr -----		78.5
----- 6 hr -----		59.8 ^{a)}
----- 9 hr -----		50.7 ^{a)}
----- 12 hr -----		46.2 ^{a)}
----- 18 hr -----		39.9 ^{a)}
----- 24 hr -----		41.5 ^{a)}
	Treated with <i>E. pisi</i> extract (----)	
Control	24 hr	81.7

Fig. 2. Effects of post-treatment of coleoptiles with the *E. pisi* extract after inoculation on penetration efficiency (PE) of *E. graminis*. a) Significantly different from the PE of the control ($P < 0.05$).

Table 3. Effects of the fractions of the *E. pisi* extract of a low or high molecular weight on penetration efficiency of *E. graminis* in coleoptile cells

Fraction	Penetration efficiency (%)	Percentage of suppression of infection ^{a)}
Low molecular weight (<10,000)	51.5 ± 3.5 ^{b)}	33.8
High molecular weight (>10,000)	74.1 ± 2.2 ^{c)}	4.8
Unfractionated	47.1 ± 4.9 ^{b)}	39.5
0.01 M CaCl ₂ (control)	77.8 ± 2.1 ^{c)}	—

a) Percentage of suppression of infection

$$= [1 - (\text{PE of treated coleoptiles} / \text{PE of control coleoptiles})] \times 100.$$

b, c) Percentages marked with different letters within a column are significantly different ($P < 0.05$).

Fig. 3. The HPLC elution profile of the *E. pisi* extract monitored by UV at 210 nm. Fractions indicated by F1 through F4 were collected and used for testing their activity to suppress the *E. graminis* infection.

Table 4. Effects of fractionated *E. pisi* extract on the *E. graminis* infection in coleoptile cells

Fraction	Concentration (mg/ml)	Penetration efficiency	Percentage of suppression of infection ^{a)}
F1	0.4	51.1 ± 4.6 ^{b)}	38.9
	0.2	73.3 ± 7.5	11.6
	0.1	78.9 ± 4.0	5.6
F2	1.0	5.1 ± 2.7 ^{b)}	93.9
	0.4	47.9 ± 3.6 ^{b)}	42.9
	0.2	85.6 ± 0.9	0
F3	2.0	73.2 ± 3.2	12.4
	1.0	78.0 ± 5.0	6.7
	0.5	83.3 ± 3.8	0
F4	0.4	32.8 ± 5.5 ^{b)}	60.8
	0.2	76.2 ± 3.7	8.9
	0.1	84.2 ± 3.6	0
Unfractionated	2.0	45.6 ± 3.9 ^{b)}	45.5
0.01 M CaCl ₂ (control)		83.6 ± 1.8	—

a) Percentage of suppression of infection

= [1 - (PE of treated coleoptiles/PE of control coleoptiles)] × 100.

b) Significantly different from PE of control (P < 0.01).

Table 5. Effects of the heat treatment of the *E. pisi* extract on its activity to suppress penetration efficiency of *E. graminis* in coleoptile cells

Heat treatment	Penetration efficiency (%)	Percentage of suppression of infection ^{a)}
Yes	51.3 ± 3.4 ^{b)}	37.9
No	42.3 ± 5.5 ^{b)}	48.8
0.01 M CaCl ₂ (control)	82.6 ± 2.6 ^{c)}	—

a) Percentage of suppression of infection

= [1 - (PE of treated coleoptiles/PE of control coleoptiles)] × 100.

b, c) Percentages marked with different letters within a column are significantly different (P < 0.05).

gave lower PEs than did the non-treated control. As indicated in Fig. 2, the 6 hr post-treatment gave 59.8% PE which was significantly lower than the control (81.7%). When the duration of treatment was prolonged, lower PEs down to about 40% were obtained. These results show that the measurable suppression of the *E. graminis* infection requires at least 6 hr post-treatment of coleoptiles with the extract.

Effects of fractionated or heated extract on the *E. graminis* infection

The stock extract solution was separated into two fractions of low (less than 10,000) and high (more than 10,000) molecular weight of both fractions was estimated by a gel-filtration standard line drawn using polyethylene glycol (PEG) of known molecular weight. As indicated by PEs in Table 3, only the

fraction of low molecular weight suppressed the *E. graminis* infection.

The stock extract solution was fractionated by means of HPLC. Based on the retention time, 4 prominent fractions, F1 through F4, as illustrated in Fig. 3 were obtained. The approximate ratio of dry weight of each fraction was F1 : F2 : F3 : F4 = 1 : 2 : 5 : 1. As indicated in Table 4, F1, F2 and F4 suppressed the *E. graminis* infection, but F3 did not. Within the concentrations of the respective fractions tested, the greatest activity was obtained by 1.0 mg/ml of F2, where the PE by *E. graminis* was drastically suppressed by 94.9%. Approximately 40–60% suppression of infection was also obtained by 0.4 mg/ml of F1, F2 and F4, and PEs were 51.1%, 47.9% and 32.8%, respectively. These PEs were significantly lower than that of control (83.6%), indicating that at least three components which are involved in suppression of the *E. graminis* infection are included in the extract preparation. As shown in Table 5, a heated extract did not lose activity to suppress the *E. graminis* infection: similar PEs were obtained in coleoptiles treated with either 2 mg/ml of the heated (51.3%) or unheated extract (42.3%).

DISCUSSION

Our previous studies^{10,14)} suggested that the maturation of appressoria of *E. pisi* might be associated with enhancement of inaccessibility in the barley coleoptile cells. Kunoh *et al.*¹⁴⁾ transferred germings of *E. graminis* from one coleoptile onto another coleoptile from which germings of *E. pisi* had been removed with a micromanipulator at or 2 hr after maturation of their appressorium. They found that the PE of transferred *E. graminis* was reduced to 50% lower than that of the control. These results suggested that *E. pisi* germings might release signal(s) that elicited the enhancement of inaccessibility by or at the time of maturation of their appressoria. A similar degree of suppression of the *E. graminis* infection was obtained by treating coleoptiles with the water soluble extract from *E. pisi* germings. Thus, it is plausible that the extract includes elicitor-like factor to enhance inaccessibility in coleoptile cells of barley. The factor putatively had a low estimated molecular weight (<10,000) and was heat-stable. The characteristics of the present factor partially resemble those that Kim and Uritani⁹⁾ separated from mycelial homogenates of *Ceratocystis fimbriata*. They obtained a water-soluble, dialyzable fraction of low molecular weight that elicited synthesis of the phytoalexin ipomeamarone in sweet potato.

At least three components which probably enhance inaccessibility of coleoptile cells are included in the present extract preparation. Among the concentrations tested, fraction 2 at 1.0 mg/ml separated by HPLC had the greatest activity to suppress the *E. graminis* infection in coleoptile cells. As the approximate ratio of dry weight of each fraction indicated, fractions 1 and 4 were quantitatively minor components in the preparation, and thus we could not test the effects of higher concentrations of these fractions on suppression of the *E. graminis* infection in this study. A further experiment is required to elucidate such effects using greater amounts of these fractions and also synergistic effects among the respective fractions.

Estimating from the retention time of the HPLC analysis and a gel-filtration standard line using PEG, it is plausible that fraction 2 has a molecular weight of 5,000–6,000. According to Tepfer and Taylor²²⁾, proteins of molecular weight up to 60,000 can permeate a substantial portion of plant cell walls. Therefore, the present elicitor-like factor could permeate coleoptile cell walls to reach internal recognition site(s) which is present putatively on the plasma membrane.

The treatment of coleoptiles with the extract after inoculation indicated that at least 6 hr was required before suppression of the *E. graminis* infection was measurable. On the other hand, the pre-treatment of coleoptiles with the extract for 1–12 hr prior to inoculation had no suppressive effect on the *E. graminis* infection. However, since *E. graminis* usually attempts penetration from its appressoria 10–12 hr after inoculation¹⁾, the present pre-treatment is not suitable to detect enhanced inaccessibility if it diminishes within 10 hr after it reaches the level required to suppress the *E. graminis* infection by about 50%. This possibility, that enhanced inaccessibility may persist only temporarily, is supported by the data of Kunoh *et al.*¹⁴⁾ which showed that the inaccessible state of coleoptile cells enhanced by *E. pisi* appressoria at maturation diminished within 24 hr after maturation of the appressoria. Similar

temporary enhancement of resistance responses and/or associated metabolic activity has been reported in tissues and cultured cells of several other plants^{4,18,19}. Appropriate experiments, such as intermittent treatments of coleoptiles with the present elicitor-like factor will be required to determine the duration of presumed enhanced inaccessibility.

As is indicated in Table 2, the extract did not affect morphogenesis of *E. graminis* up to penetration attempt. Since 40% of *E. graminis* can produce a haustorium even in the presence of the extract in coleoptiles, that the extract directly interferes with haustorium formation may have a less possibility. However, because we could not test haustorium formation of *E. graminis* in *in vitro* system without host resistance responses, we can not rule out the possibility that the preparation directly inhibits the process of haustorium formation, rather than enhancing inaccessibility of coleoptile cells. A further experiment will be required to clarify this possibility.

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Literature cited

1. Aist, J.R. and Israel, H.W. (1977). Papilla formation: timing and significance during penetration of barley coleoptiles by *Erysiphe graminis hordei*. *Phytopathology* 67: 455-461.
2. Aist, J.R. and Israel, H.W. (1986). Autofluorescent and ultraviolet-absorbing components in cell walls and papilla of barley coleoptiles and their relationship to disease resistance. *Can. J. Bot.* 64: 266-272.
3. Bushnell, W.R. (1981). Incompatibility conditioned by the *Mla* gene in powdery mildew of barley: the halt in cytoplasmic streaming. *Phytopathology* 59: 1141-1146.
4. Chai, H.B. and Doke, N. (1987). Activation of the potential of potato leaf tissue to react hypersensitively to *Phytophthora infestans* by cystospore germination fluid and the enhancement of this potential by calcium ions. *Physiol. Mol. Plant Pathol.* 30: 27-37.
5. Ebel, J. (1986). Phytoalexin synthesis: The biochemical analysis of the induction process. *Ann. Rev. Phytopathol.* 24: 235-264.
6. Hoppe, H.H., Hume, H. and Heitefuss, R. (1980). Elicitor induced accumulation of phytoalexins in healthy and rust infected leaves of *Phaseolus vulgaris*. *Phytopathol. Z.* 97: 85-88.
7. Keen, N.T. (1975). Specific elicitors of phytoalexin production: Determinants of race specificity in pathogens? *Science* 187: 74-75.
8. Keen, N.T. and Legrand, M. (1980). Surface glycoprotein: evidence that they may function as the race specific phytoalexin elicitors of *Phytophthora megasperma* f. sp. *glycinea*. *Physiol. Plant Pathol.* 17: 175-192.
9. Kim, W.K. and Uritani, I. (1974). Fungal extracts that induce phytoalexins in sweet potato roots. *Plant Cell Physiol.* 15: 1093-1098.
10. Kobayashi, I., Komura, T., Sakamoto, Y., Yamaoka, N. and Kunoh, H. (1990). Recognition of a pathogen and a nonpathogen by barley coleoptile cells. (I). Cytoplasmic responses to the nonpathogen, *Erysiphe pisi*, prior to its penetration. *Physiol. Mol. Plant Pathol.* 37: 479-490.
11. Kunoh, H. (1982). Primary germ tubes of *Erysiphe graminis* conidia. In *Plant Infection: The Physiological and Biochemical Basis* (Asada, Y., Bushnell, W.R., Ouchi, S. and Vance, P. eds.). Japan Sci. Soc. Press, Tokyo/Springer-Verlag, Berlin. pp. 45-59.
12. Kunoh, H., Hayashimoto, A., Harui, M. and Ishizaki, H. (1985). Induced susceptibility and enhanced resistance at cellular level in barley coleoptiles. I. The significance of timing of fungal invasion. *Physiol. Plant Pathol.* 27: 43-54.
13. Kunoh, H., Katsuragawa, N., Yamaoka, N. and Hayashimoto, A. (1988). Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. III. Timing and localization of enhanced inaccessibility in a single coleoptile cell and its transfer to an adjacent cell. *Physiol. Plant Pathol.* 33: 81-93.
14. Kunoh, H., Komura, T., Kobayashi, I. and Yamaoka, N. (1990). Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. VII. Enhancement of inaccessibility at the pre-penetration stage of a nonpathogen. *Physiol. Mol. Plant Pathol.* 37: 399-407.
15. Kunoh, H., Toyoda, K., Yamaoka, N. and Kobayashi, I. (1989). Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. V. Duration of stimulus by non-pathogen in relation to enhanced inaccessibility. *Physiol. Mol. Plant Pathol.* 35: 507-518.

16. Kunoh, H., Toyoda, K., Yamaoka, N. and Kobayashi, I. (1992). Morphogenesis of *Erysiphe pisi* conidia on artificial substrata. Trans. Mycol. Soc. Japan 33: 87-93.
17. Mayama, S., Tani, T., Matsuura, Y., Ueno, T. and Fukami, H. (1981). The production of phytoalexins by oat in response to crown rust, *Puccinia coronata* f. sp. *avenae*. Physiol. Plant Pathol. 19: 217-226.
18. Ryder, T.B., Cramer, C.L., Bell, J.N., Robbins, M.P., Dixon, R.A. and Lamb, C.J. (1984). Elicitor rapidly induces chalcone synthase mRNA in *Phaseolus vulgaris* cells at onset of the phytoalexin defense response. Proc. Natl Acad. Sci., USA 84: 5520-5524.
19. Schmelzer, E., Borner, H., Grisebach, H., Ebel, J. and Hahlbrock, K. (1984). Phytoalexin synthesis in soybean (*Glycine max*). Similar time courses of mRNA induction in hypocotyls infected with a fungal pathogen and in cell cultures treated with fungal elicitor. FEBS lett. 172: 59-63.
20. Takamatsu, S., Ishizaki, H. and Kunoh, H. (1978). Cytological studies of early stages of powdery mildew in barley and wheat. V. Effect of calcium on the infection of coleoptile of barley by *Erysiphe graminis hordei*. Can. J. Bot. 56: 2544-2549.
21. Tani, T., Yamamoto, H., Onoe, T. and Naito, N. (1975). Initiation of resistance and host cell collapse in the hypersensitive reaction of oat leaves against *Puccinia coronata avenae*. Physiol. Plant Pathol. 7: 231-242.
22. Tepfer, M. and Taylor, I.E.P. (1981). The permeability of plant cell walls as measured by gel filtration chromatography. Science 213: 761-763.
23. West, C.A. (1981). Fungal elicitors of the phytoalexin response in higher plants. Naturwissenschaften 68: 447-457.
24. Yamaoka, N., Lyons, P.C., Hipskind, J. and Nicholson, R.L. (1990). Elicitor of sorghum phytoalexin synthesis from *Colletotrichum graminicola*. Physiol. Mol. Plant Pathol. 37: 255-270.

和 文 摘 要

豊田和弘・白石友紀・小林一成・山岡直人・久能 均：エンドウうどんこ病菌 *Erysiphe pisi* 発芽胞子の水抽出液はオオムギ子葉鞘上のオオムギうどんこ病菌 *E. graminis* の感染を抑制する

オオムギに対して非病原菌である *Erysiphe pisi* 発芽胞子表層から水抽出した物質でオオムギ子葉鞘を処理し、接種した病原菌 *E. graminis* の感染に及ぼす影響を検討したところ、約50%の抑制率が得られた。この抑制効果は、抽出物中の低分子、耐熱性の成分に依存すると考えられた。抽出物を HPLC で分画したところ四つのピークが得られ、そのなかの一つの画分が *E. graminis* の感染を約95%抑制した。抽出物は *E. graminis* 胞子の発芽から侵入までの過程を阻害しなかった。本菌の吸器形成を検定する *in vitro* 系がないので、侵入から吸器形成するまでの過程に及ぼす本抽出物の影響を直接検定することはできなかったが、抽出物で処理した子葉鞘でも約40%の吸器形成率が得られるので抽出物が直接抗菌性を示している可能性は低い。したがって、本抽出物には子葉鞘の拒否性を高めるエリシター活性があると考えられた。