Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 67 (2006) 1104–1109
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

# Changes in lipid composition of *Blumeria graminis* f.sp. *tritici* conidia produced on wheat leaves treated with heptanoyl salicylic acid

Jérôme Muchembled, Anissa Lounès-Hadj Sahraoui *, Anne Grandmougin-Ferjani, Michel Sancholle

*Laboratoire de Mycologie/Phytopathologie/Environnement, Université du Littoral Côte d'Opale, 17 Avenue Blériot, BP 699, 62228 Calais Cedex, France*

Received 5 July 2005; received in revised form 21 February 2006
Available online 2 May 2006

**Abstract**

Treatment of wheat leaves with heptanoyl salicylic acid (HS) and trehalose at concentrations of 0.1 and 15 g $l^{-1}$, prior to fungal inoculation, resulted in 40% and 60% protection, respectively, against powdery mildew. The total lipid composition of *Blumeria graminis* f.sp. *tritici* (*Bgt*) conidia, the causal agent of wheat powdery mildew, was compared when produced on wheat leaves, respectively, untreated and treated with the two elicitors, HS and trehalose. An obvious effect was observed on lipid composition (sterol and fatty acid (FA)) of *Bgt* conidia produced on wheat leaves treated with HS. A total of 16 FA ($C_{12}$–$C_{24}$ saturated and unsaturated) as well as unusual methoxylated Fatty Acids (mFA) (3-methoxydocosanoic and 3-methoxytetracosanoic acids) were detected in the conidia. Medium chain FA were predominant in HS treated conidia (64.65%) while long chain fatty acids constituted the major compounds in untreated conidia (62%). The long chain/medium chain FA ratio decreased from 1.8 in the conidia produced on untreated leaves to 0.5 in the conidia obtained from HS treated leaves. When comparing the sterol composition of *Bgt* conidia produced on leaves treated with HS versus conidia obtained from untreated ones, very important changes within the two major classes can be seen. In particular, 24-methylsterols, e.g., 24-methylenecholesterol and 24-methylcholesta-7,24-dien were reduced by about 82% whereas 24-ethylsterols, e.g., 24-ethylcholesterol and 24-ethylcholesta-5,22-dienol were increased by about 85%. The 24-methylsterols/24-ethylsterols ratio was reduced by ninefold in the conidia produced from HS treated leaves.


*Keywords:* *Blumeria graminis*; *Triticum aestivum* (var. sideral); Lipid, fatty acid; Sterols; Wheat; Powdery mildew; Trehalose; Heptanoyl salicylic acid

## 1. Introduction

Cereal powdery mildew is a disease causing important yield diminution in barley, oat, and wheat worldwide. The causal agent is *Blumeria graminis* f.sp. *tritici* (*Bgt*) (syn. *Erysiphe graminis*), a biotrophic foliar pathogen. Since the 1970s, powdery mildews have been controlled mainly by fungicides which inhibit the P450 cytochrome 14α-demethylase step in sterol biosynthesis (demethylase inhibitors, DMIs) (Köller, 1992). Because of the use of DMIs, the sterol composition of powdery mildews has been well studied. The sterol compositions of barley powdery mildew (*Blumeria graminis* f.sp. *hordei*, Bgh), cucurbit powdery mildew (*Sphaerotheca fuliginea*) and apple powdery mildew (*Podosphaera leucotricha*) have been analyzed by Loeffler et al. (1984) and that of wheat powdery mildew (Bgt) by Senior et al. (1995), Engels and De Waard (1998) and Muchembled et al. (2000). These fungi are atypical since they do not contain ergosterol which is usually the principal sterol in most fungi (Weete, 1980). Only two main sterols were previously identified in wheat, barley, cucurbit and apple powdery mildews, ergosta-5,24(24)[1]dienol (24-methylenecholesterol) as the major component and

*Abbreviations:* *Bgt*, *Blumeria graminis* f.sp.*tritici*; f.sp., formae specialis; FA, fatty acids; mFA, methoxylated fatty acids; HS, heptanoyl salicylic acid; SA, salicylic acid; HA, heptanoyl acid.

* Corresponding author. Tel.: +33 03 21 96 48 07; fax: +33 03 21 34 71 13.
*E-mail address:* lounes@univ-littoral.fr (A.Lounès-Hadj Sahraoui).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.02.025

ergosta-7,24(24)[1]-dienol (episterol), an intermediate in fungal sterol biosynthesis (Mercer, 1991). Additionally, cholesterol represented around 15% of the total sterol in conidia of cucurbit powdery mildew (Loeffler et al., 1992).

Although secondary effects of fungicides on FA composition of several fungi have been reported (Weete et al., 1983), little research concerning the FA composition of powdery mildew has been completed. A total of 19 FA ($C_{12}$–$C_{24}$ saturated and unsaturated) as well as unusual methoxylated fatty acids (3-methoxydocosanoic and 3-methoxytetracosanoic acids) were detected in *Bgt* conidia (Muchembled et al., 2005). It was also shown that the total lipid composition (sterol and FA) correlates strongly with the age of *Bgt* conidia (Muchembled et al., 2000, 2005). On the other hand, studies in recent years demonstrated that lipoxygenases (LOXs; EC 1.13.11.12), non-heme iron-containing dioxygenases catalysing the dioxygenation of polyunsaturated fatty acids containing a (*Z*,*Z*)-1,4-pentadiene moiety (Helena and Mario, 2002), might play an important role in the plant defence response. Recently, Shah (2005) reported the involvement of lipid metabolism in plant disease resistance. *Bgt* being an obligate biotrophic pathogen, changes in the plant lipids treated with elicitor could ultimately have an effect on the fungal lipid composition. To our knowledge, the effect of elicitors on fungal lipid composition has never been reported.

In the present paper, analysis of the lipid (sterol and FA) compositions of *Bgt* conidia produced on wheat leaves treated with two elicitors (HS and trehalose) was carried out.

The stimulation of the wheat defence reactions was obtained by using an authentic compound, HS (synthesized by esterification of 2-OH benzoic with heptanoic acid) and trehalose, a non-reducing disaccharide commonly found in a wide variety of organisms including fungi. These two molecules were used because they have two main characteristics; first, they are non-toxic to the environment and the plant, and secondly, they confer a good protection level. In fact, the present work follows an attempt to isolate naturally occurring molecules that could be used in the control of plant diseases as an alternative to classical chemical fungicides. Previous studies showed that salicylic acid (SA) and trehalose are inducers of plant defences (Pierpoint, 1994; Reignault et al., 2001).

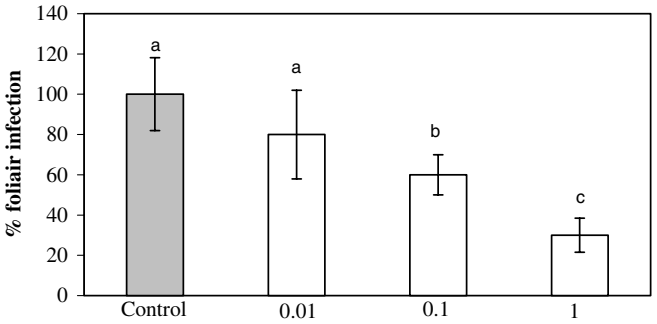


Fig. 1. Effect of different concentrations of HS (g $l^{-1}$) on the percentage of infection of wheat leaves by *Bgt*. Control has been treated with sterile water supplemented with ethanol 30% (v/v) + Citowett 0.025% (v/v). Data represent means from three experiments and bars with the same letters are not significantly different ($P < 0.05$). Each experiment consisted of 50 leaves.

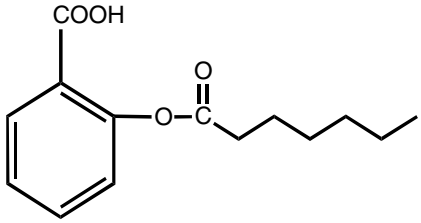


Fig. 2. Structure of HS (2-OH benzoic acid esterified by heptanoic acid).

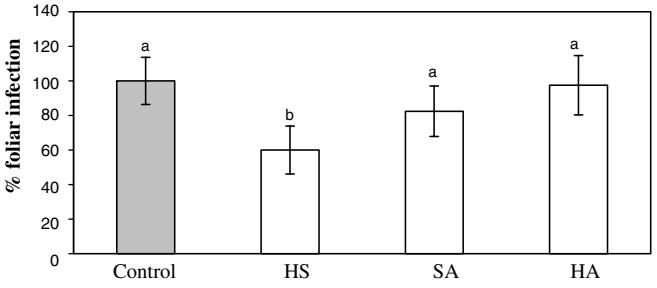


Fig. 3. Effect of HS (0.1 g $l^{-1}$), SA, and HA (400 μM) on the percentage of infection of wheat leaves by *Bgt*. Control has been treated with sterile water supplemented with ethanol 30% (v/v) + Citowett 0.025% (v/v). Data represent means from three experiments and bars with the same letters are not significantly different ($P < 0.05$). Each experiment consisted of 50 leaves. HS, heptanoyl salicylic acid; SA, salicylic acid; HA, heptanoic acid.

## 2. Results and discussion

Several concentrations of HS and trehalose were tested and the protections conferred on wheat recorded. Treatment on wheat leaves with HS at concentrations of 0.01, 0.1 and 1 g $l^{-1}$, prior to fungal inoculation, resulted in 20%, 40% and 70% protection, respectively, against the powdery mildew of wheat (Fig. 1). HS (2-OH benzoic acid esterified by heptanoic acid (HA)), (Fig. 2), a SA derivative, was twice (40%) as efficient as SA (20%) when used on its own (Fig. 3). HA did not reduce the infection and indeed probably improved the effect of SA by increasing its penetration through the hydrophobic plant cuticle. If so, protection should be related to the mode of action of SA which acts by itself as an inducer of plant defence responses by inhibiting catalase activity, leading to an increased level of hydrogen peroxide (Chen et al., 1993; Vernooîj et al., 1994).

By using different concentrations of trehalose (1, 5, 10, 15 g $l^{-1}$), reductions of infection level of 0%, 0%, 50% and 60% were, respectively, observed (Fig. 4). We suggest that an hydrophilic molecule such as trehalose cannot penetrate the hydrophobic plant cuticle very efficiently and

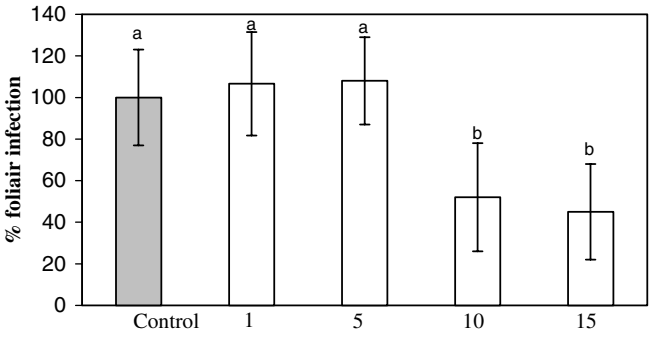


Fig. 4. Effect of different concentrations of trehalose ($g\,l^{-1}$) on the percentage of infection of wheat leaves by *Bgt*. Control has been treated with sterile water supplemented with Citowett 0.025% (v/v). Data represent means from three experiments and bars with the same letters are not significantly different ($P < 0.05$). Each experiment consisted of 50 leaves.

that spraying more concentrated solutions could compensate for this effect. Trehalose is a highly hydrophilic molecule and the importance of the extent of its penetration through the plant cuticle was supported by the experiments of immersing the basal end of detached leaves in trehalose solution described by Reignault et al. (2001).

Concerning trehalose, it was demonstrated in previous studies that this molecule was non-toxic to the fungus and that the protection conferred by trehalose is the result of induced resistance. In fact, it appeared clearly that trehalose had no direct effect on the fungus during penetration but that the epidermal cell response to fungal invasion, that is papilla deposition, was enhanced. In addition, phenylalanine ammonia-lyase and peroxidase activities, both parts of the phenylpropanoid pathway leading to the synthesis of lignin but also to other secondary metabolites such as phenolics that could contribute as well as to autofluorescence of papilla (Ride and Barber, 1987) were enhanced (Reignault et al., 2001).

Table 1 summarizes the results of the sterol composition (percentage of total sterols) of *Bgt* conidia produced on untreated (control) and wheat leaves treated with HS ($0.1\,g\,l^{-1}$).

In the conidia produced on untreated wheat leaves (control), the major sterol was 24-methylenecholesterol (37.5%) followed by 24-ethylcholesterol (31%). Small amounts of 24-ethylcholesta-5,24-dienol (11%), 24-methylcholesta-7,24-dienol (5.7%), 24-ethylcholesta-5,22-dienol (5%) and cholesterol (1.3%) were detected. Ergosterol was absent from *Bgt* conidia. This result is in agreement with those reported by Weete (1980).

When comparing the sterol composition of *Bgt* conidia produced on leaves treated with HS to that of conidia obtained from untreated ones, very important changes within the two major classes can be seen. In particular, 24-methylsterols, e.g., 24-methylenecholesterol and 24-methylcholesta-7,24-dienol were reduced by about 82% whereas 24-ethylsterols, e.g., 24-ethylcholesterol and 24-ethylcholesta-5,24-dienol were increased by about 85%.

Table 1
Sterol composition (percentage of total sterols) of *Bgt* conidia produced on untreated (control) and treated wheat leaves with HS $0.1\,g\,l^{-1}$

| | *Bgt* conidia | |
|---|---|---|
| | Control | Treated |
| Cholesterol | $1.30 \pm 0.10$ | $1.70 \pm 0.15$ |
| 24-Methylenecholesterol | $37.50 \pm 4.00$ | $7.00^{a} \pm 1.05$ |
| 24-Ethylcholesta-5,22-dienol | $5.00 \pm 0.80$ | $8.30^{a} \pm 0.40$ |
| 24-Methylcholesta-7,24-dienol | $5.70 \pm 0.70$ | $1.00^{a} \pm 0.50$ |
| 24-Ethylcholesterol | $31.00 \pm 3.90$ | $52.00^{a} \pm 3.00$ |
| 24-Ethylcholesta-5,24-dienol | $11.00 \pm 2.10$ | $20.00^{a} \pm 0.70$ |
| Other sterols | $8.50 \pm 1.10$ | $10.00 \pm 1.45$ |
| Ratio 24-methylsterols/24-ethylsterol | 0.90 | 0.10 |
| Total sterol content $\mu g\,mg^{-1}$ dry weight | $12.30 \pm 0.50$ | $18.00^{a} \pm 0.11$ |

Data represent means of three experiments.
[a] Indicates significant difference compared to control.

The 24-methylsterols/24-ethylsterols ratio decreased from 0.9 in the conidia produced on untreated leaves to 0.1 in the conidia obtained from treated leaves. Moreover, this qualitative evolution of the sterol composition of *Bgt* conidia was coupled with a quantitative evolution. This amount of sterols was about $12.3\,\mu g\,mg^{-1}$ dry weight in the conidia produced on untreated leaves and increased to $18\,\mu g\,mg^{-1}$ dry weight in conidia produced on HS treated wheat leaves.

Table 2 shows the qualitative and quantitative FA compositions of *Bgt* conidia produced on untreated (control) and wheat leaves treated with HS. Sixteen FAs were detected, long chain acids ($C_{22}$ and $C_{24}$) being the major ones. Unusual long chain monoenoic FAs were detected and identified as 3-methoxydocosanoic and methoxytetracosanoic acids in *Bgt* conidia, using deuterated reagent and GC–MS according to Muchembled et al. (2005).

Significant modifications of the FA composition were detected in conidia produced on leaves treated with HS. C16:0, C18:0 and C18:1 increased by 2,57, 3,2 and 2,4 fold respectively, whereas the C22:1, C22:0 3,$OCH_3$, C24:0, C24:1 and C24:0 3,$OCH_3$ were reduced by 2; 1,9; 2,5; 2,72 and 2,46 fold respectively. A majority of medium chain are present in conidia produced on treated leaves whereas the long chain FA were predominant in conidia produced on untreated leaves. The long chain/medium chain FA ratio decreased from 1.8 in the conidia produced on untreated leaves to 0.5 in conidia obtained from treated ones, indicating that the FA composition was affected by HS spraying. The proportions of methoxylated long chains decreased in conidia produced on treated wheat leaves by 55%. The amount of total FA was about $21.3\,\mu g\,mg^{-1}$ of dry weight in conidia produced on untreated leaves and decreased clearly to $9\,\mu g\,mg^{-1}$ of dry weight in conidia from HS treated.

Tables 3 and 4 summarize the total fatty acid and sterol composition of *Bgt* conidia obtained from untreated (control) or wheat leaves treated with trehalose ($15\,g\,l^{-1}$).

When comparing the relative lipid (sterol and FA) composition of *Bgt* conidia produced on leaves treated with tre-

Table 2
Fatty acid composition (percentage of total fatty acids) of *Bgt* conidia produced on untreated (control) and treated wheat leaves with HS (0.1 g $l^{-1}$)

| | *Bgt* conidia | |
|---|---|---|
| | Control | Treated |
| C12:0 | tr | 1.25 ± 0.07 |
| C14:0 | tr | 0.80 ± 0.02 |
| C16:0 | 7.00 ± 0.10 | 18.00[a] ± 0.07 |
| C16:1 | tr | 1.00 ± 0.40 |
| C18:0 | 5.00 ± 0.02 | 16.00[a] ± 0.64 |
| C18:1 (*trans*) | 3.50 ± 0.10 | 8.30[a] ± 0.15 |
| C18:2 (*cis*/*cis*) | 13.50 ± 1.40 | 16.30 ± 0.65 |
| C18:3 | 5.00 ± 0.55 | 3.00 ± 0.10 |
| C22:0 | 8.50 ± 1.80 | 5.00 ± 0.30 |
| C22:1 (*cis* + *trans*) | 3.50 ± 0.08 | 4.50 ± 0.00 |
| C22:1 | 4.00 ± 0.15 | 2.00[a] ± 0.07 |
| C22:0 3,$OCH_3$ | 11.50 ± 0.30 | 6.00[a] ± 1.70 |
| C24:0 | 5.00 ± 0.60 | 2.00[a] ± 0.07 |
| C24:1 (*cis* + *trans*) | 5.00 ± 0.30 | 4.00 ± 0.25 |
| C24:1 | 6.00 ± 0.25 | 2.20[a] ± 0.00 |
| C24:0 3,$OCH_3$ | 18.50 ± 0.25 | 7.50[a] ± 1.77 |
| Ratio lc/mc | 1.80 | 0.50 |
| % MethoxyFA | 30.00 | 13.50 |
| Total content μg $mg^{-1}$ dry weight | 21.30 ± 0.25 | 9.00[a] ± 0.70 |

Data represent means of three experiments.
tr, trace (<1%).
lc/mc = long chain ($C_{22}$–$C_{24}$)/medium chain ($C_{12}$–$C_{18}$).
[a] Indicates significant difference compared control.

halose and untreated leaves (control), no significant differences can be detected. Contrarily to HS, trehalose does not affect the lipid composition *of Bgt* conidia.

The assessment of protection against wheat powdery mildew by using HS indicated a metabolic modification in treated seedlings. An obvious effect on lipid (sterol and FA) composition of *Bgt* conidia produced on HS treated wheat leaves was observed.

These changes in conidia lipid profile as a result of treatment with HS seem to be linked to a modified physiological state of the fungus.

In fact, similar changes in the lipid (sterol and FA) composition of *Bgt* conidia were reported by Muchembled et al. (2000, 2005) when the total lipid composition of the causal agent of wheat powdery mildew, was analyzed as a function of their age. It was shown that the total lipid composition (both sterols and FA) was strongly correlated with the age of *Bgt* conidia. Medium chain FA were predominant in young conidia (75%, including 13% of mFA) while long chain FA constituted the major compounds in old conidia (74%, including 30% of mFA). Predominance of medium chain FA in young conidia and long chain ones in old conidia may result from a significant elongation of FA. Medium and long chain FA may possibly have different physiological functions in relation to the ontogeny of *Bgt* conidia (Muchembled et al., 2005). Similar results have also been described in *Saccharomyces cerevisiae* (Welch and Burligame, 1973) and in *Neurospora crassa* (McKeon et al., 1997).

It was also demonstrated that sterol composition was greatly modified during the ontogeny of *Bgt* conidia. In particular, 24-methylsterols, e.g., 24-methylenecholesterol and episterol, are the major sterols in old conidia whereas 24-ethylsterols, e.g., 24-ethylcholesta-5,22-dienol, 24-ethylcholesterol and Δ5-avenasterol, are the main sterols in young conidia (Muchembled et al., 2000).

The qualitative as well as quantitative changes observed in lipid compositions (both sterol and FA) of *Bgt* conidia produced on HS treated wheat leaves resembled the modifications observed in young *Bgt* conidia. It seems that the modifications detected in lipid composition of *Bgt* conidia obtained from HS treated wheat leaves could be a result of a delayed development of the pathogen, provoked by the defence reactions set up by the plant-host. The protection induced by HS in wheat leaves probably resulted from stimulation of various defence reactions leading to the formation of physicochemical barriers which prevented the penetration of some conidia. This is corresponding to the % protection recorded. The remaining conidia achieved

Table 3
Sterol composition (percentage of total sterols) of *Bgt* conidia obtained from untreated (control) or treated wheat leaves with trehalose (15 g $l^{-1}$)

| | *Bgt* conidia | |
|---|---|---|
| | Control | Treated |
| Cholesterol | 2.00 ± 0.70 | 0.50 ± 0.07 |
| 24-Methylenecholesterol | 83.00 ± 5.60 | 86.00 ± 0.35 |
| 24-Ethylcholesta-5,22-dienol | 1.00 ± 0.35 | 0.10 ± 0.10 |
| 24-Methylcholesta-7,24-dienol | 5.00 ± 1.50 | 8.50 ± 0.35 |
| 24-Ethylcholesterol | 6.00 ± 3.40 | 2 .00 ± 0.50 |
| 24-Ethylcholesta-5,24-dienol | 1.50 ± 0.30 | 0.50 ± 0.00 |
| Other sterols | 1.50 ± 0.50 | 2.00 ± 0.80 |
| Total content μg $mg^{-1}$ dry weight | 9.25 ± 0.30 | 7.65 ± 0.30 |

Data represent means of three experiments.

Table 4
Total fatty acid composition of *Bgt* conidia obtained from untreated (control) or treated wheat leaves with trehalose (15 g $l^{-1}$)

| | *Bgt* conidia | |
|---|---|---|
| | Control | Treated |
| C12:0 | nd | nd |
| C14:0 | nd | nd |
| C16:0 | 2.70 ± 0.50 | 3.70 ± 0.00 |
| C16:1 | tr | tr |
| C18:0 | tr | tr |
| C18:1 (*trans*) | tr | tr |
| C18:2 (*cis*/*cis*) | 9.65 ± 3.50 | 12.95 ± 0.60 |
| C18:3 | 4.50 ± 2,45 | 6.00 ± 0.20 |
| C22:0 | 12.10 ± 2.75 | 10.15 ± 2.00 |
| C22:1 (*cis* + *trans*) | 4.00 ± 1.80 | 2.40 ± 0.80 |
| C22:1 | 11.65 ± 2.30 | 9.80 ± 2.00 |
| C22:0 3,$OCH_3$ | 8.00 ± 0.65 | 5.90 ± 2.25 |
| C24:0 | 6.50 ± 0.45 | 7.00 ± 1.55 |
| C24:1 (*cis* + *trans*) | 6.70 ± 0.40 | 4.45 ± 3.50 |
| C24:1 | 19.20 ± 1.40 | 19.70 ± 4.80 |
| C24:0 3,$OCH_3$ | 14.00 ± 2.30 | 17.40 ± 4.10 |
| Total content μg $mg^{-1}$ dry weight | 18.60 ± 1.80 | 23.40 ± 1.55 |

Data represent means of three experiments.
nd, not detected; tr, trace (<1%).

their penetration in the plant with some delay. Consequently, the conidia produced on wheat leaves treated with HS produced less growth. So, the conidia obtained from treated wheat leaves were probably younger than the ones produced on untreated leaves.

## 3. Experimental

### 3.1. Plant and fungal material

Seeds of wheat (*Triticum aestivum* L.) cv. Sideral, a variety without any known resistance gene to any *B. graminis* f.sp. *tritici* race and highly susceptible to powdery mildew were sown in $30 \times 12$ cm plastic trays. Plants were germinated and grown in compost for 2 weeks to complete expansion of the first two primary leaves in a Phytotron (250 μmol $m^{-2}$ $s^{-1}$, 14 h light, 10 h dark, 90% rh, day temperature 18 °C, night temperature 10 °C).

The *B. graminis* f.sp. *tritici* MPEbgt1 isolate used in this study was kindly provided by Dr. M.-P. Latorse from Bayer Crop Science (Lyon, France). The fungus was inoculated and maintained on Sideral plants in a separate Phytotron. Heavily sporulating leaves were shaken above two-weeks-old plants and inoculated plants were left in the Phytotron until new fully sporulating leaves were obtained, usually within 10 days.

### 3.2. Chemicals

Unless otherwise stated, the different chemicals used in this study were purchased from Sigma (Saint-Quentin Fallavier, France).

The synthesized HS (Fig. 2) (99.9% purity) was kindly provided by Dr. D. Couturier (Molecular Ingineering Laboratory, USTL 59655 Villeneuve d'Ascq Cedex, France).

Trehalose (99% purity) was a gift from Lesaffre (Marcq-en-Baroeul, France). 'Fluorinert' FC43 was obtained from Minnesota Mining and Manufacturing (Saint Paul, Minnesota, USA). Citowett was purchased from B.A.S.F. (Levallois-Perret, France).

### 3.3. Statistical analysis

ANOVA were carried out with the statistical program STATIGRAPHICS release 4.0 (Manugistic, Inc., Rockville, MD, USA) to compare values obtained in the different experiments. The method used to discriminate among the means was the Student–Newman–Keuls multiple comparison procedure ($P < 0.05$).

### 3.4. Plant treatment and inoculation, infection level assessment

Trehalose and HS solutions were freshly made before use. Trehalose was prepared in sterile water supplemented with 0.025% (v/v) Citowett, a wetting agent. The synthesized HS (Fig. 1) was solubilized in ethanol 30% (v/v) and supplemented with Citowett 0.025% (v/v) Trehalose and HS treatments consisted of spraying two-weeks-old whole plants with solution at 15 and 0.1 g $l^{-1}$, respectively. Trehalose and HS controls were, respectively, plants sprayed with sterile water with addition of Citowett 0.025% (v/v) or ethanol 30% (v/v) + Citowett 0.025% (v/v).

Inoculation of the plant material was performed 48 h after trehalose and SH treatments by spraying with a suspension of *Bgt* conidia in Fluorinert (FC43) solution ($5 \times 10^5$ conidia $ml^{-1}$ FC43) resulting in applying approx. 40 conidia $cm^{-2}$ leaf area. At the time of inoculation, the wheat plants were 14 days old.

When symptoms arise (usually 8 days after inoculation), we first begin by the assessment of the infection level on untreated (control) and leaves treated with trehalose and HS. The infection level of plant material inoculated with *Bgt* was recorded by assessing the number of sporulating colonies on treated material compared with controls, as soon as symptoms were visible. Powdery mildew colonies were counted when still separate and before coalescence occurred.

In the second experiment, the leaves (10 cm long) were detached and then placed horizontally in agar plates ($12 \times 12$ cm, 8 leaves per box) by immersing the basal and the top ends into 0.75% (w/v) agar supplemented with benzimidazole (10 mg $l^{-1}$) to prevent senescence. The plates containing detached untreated (control) and wheat leaves treated with trehalose and HS were placed in a growth chamber, the temperature of which was maintained at 18 °C (photoperiod: 14–10 h; white light: 250 μmol $m^2$ $s^{-1}$). Conidia of *Bgt* were harvested 12 days after inoculation by gentle shaking of the leaves above aluminium foil. The conidia used originated from separate groups of leaves inoculated simultaneously and grown in the same conditions. Being biotrophic, powdery mildews are very difficult to recover, particularly because their sporulation in vitro is low. About 80 leaves were used for the production of conidia. The harvested conidia were exclusively the conidia which detached spontaneously, without any contaminations by fragments of leaves. They were stored at −80 °C prior to freeze-drying and lipid analysis.

### 3.5. Sterol extraction, analysis and identification

The freeze-dried *Bgt* conidia were extracted and analyzed as previously reported by Grandmougin-Ferjani et al. (1999) except that the steryl acetates were analyzed by GC-FID equipped with a glass capillary column (DB5 J&W, 25 m × 0.25 mm i.d., film thickness 0.25 μm, $H_2$ 1.2 ml $min^{-1}$). Steryl acetates were quantified using free cholesterol as an internal standard and sterol structures were identified by GC–MS at an ionizing potential of 70 eV. Data were compared with reference compounds and those reported in the paper of Rahier and Benveniste (1989). Sterol analyses of conidia were performed in triplicate.

### 3.6. Fatty acid extraction, analysis and identification

The freeze-dried *Bgt* conidia produced were extracted (3×) using 3 ml $CH_2Cl_2$/MeOH (2:1, v/v) at 70 °C according to Grandmougin-Ferjani et al. (1999). The total lipid extract was saponified with 1 ml of 6% (w/v) methanolic KOH for 1 h at 90 °C. After addition of 1 ml water, the non-saponifiable fraction was extracted (3×) with 3 ml of hexane prior to extraction of total FA. The *aqueous* phase was adjusted to pH 1 with concentrated HCl. FA were extracted (3×) in 5 ml hexane and dried over anhydrous $Na_2SO_4$. After evaporation under $N_2$, FA were methylated using 1 ml of $BF_3$/MeOH (14%) according to Morrison and Smith (1964). Analysis and quantification of the mFA were performed as described by Muchembled et al. (2005). They were analyzed by a GC-FID equipped with a glass capillary column (FFAP, 30 m × 0.25 mm id, $H_2$ 1.2 ml $min^{-1}$). The temperature program was: 60–160 °C (20 °C $min^{-1}$), 160–195 °C (1 °C $min^{-1}$), 195–230 °C (2.5 °C $min^{-1}$) and finally 230 °C for 10 min. mFA were quantified using eicosanoic acid methyl ester ($C_{21:0}$) as an internal standard. GC–MS identification was performed on a Shimadzu GC17A chromatograph linked to a HS-QP5000 mass spectrometer (70 eV), using a 30 m × 0.22 mm id column coated with bpx-70. The carrier gas was helium (8 ml $min^{-1}$). Column temperature was programmed from 180 to 300 °C (3 °C $min^{-1}$). FA analyses of conidia were performed in triplicate.

## Acknowledgements

This work was supported by a grant from Nord-Pas de Calais Region, France.

The authors thank Dr. D. Couturier for obtaining heptanoyl salicylic acid (Dr. D. Couturier, Molecular Engineering Laboratory, USTL 59655 Villeneuve d'Ascq Cedex, France), Dr. Y. Tierny and Dr. S. Guyard from U.S.T.L. (France) for kindly allowing us to use their GC–MS. We are very grateful to Dr. Dorothy Lösel (University of Sheffield) for having carefully read our text and edited language errors.

## References

Chen, Z., Silva, H., Klessig, D.F., 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science 262, 1883–1886.

Engels, A.J.G., De Waard, M.A., 1998. Sterol composition of isolates of *Erysiphe graminis* f.sp. *tritici* differing in sensitivity to fenpropimorph. Pest. Sci. 52, 258–262.

Grandmougin-Ferjani, A., Dalpé, Y., Hartmann, M.A., Laruelle, F., Sancholle, M., 1999. Sterol distribution in arbuscular mycorrhizal fungi. Phytochemistry 50, 1027–1031.

Helena, P., Mario, R.S., 2002. Plant lipoxygenases. Physiological and molecular features. Plant Physiol. 130, 15–21.

Köller, W., 1992. Antifungal agents with target sites in sterol functions and biosynthesis. In: Köller, W. (Ed.), Target Sites of Fungicides Action. Academic Press, Boca Raton, pp. 119–206.

Loeffler, R.S.T., Butters, J.A., Hollomon, D.W., 1984. The lipid content of powdery mildews and their sensitivity to fungicides. British Crop Protection Conference – Pests and Diseases 9A–12, 911–916.

Loeffler, R.S.T., Butters, J.A., Hollomon, D.W., 1992. The sterol composition of powdery mildews. Phytochemistry 31, 1561–1563.

McKeon, T.A., Goodrich-Tanrikulu, M., Lin, J.T., Stafford, A., 1997. Pathways for fatty acid elongation and desaturation in *Neurospora crassa*. Lipids 32, 1–5.

Mercer, E.I., 1991. Sterol biosynthesis inhibitors: their current status and modes of action. Lipids 26, 584–597.

Morrison, W.R., Smith, L.M., 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5, 600–608.

Muchembled, J., Lounès-Hadj Sahraoui, A., Grandmougin-Ferjani, A., Sancholle, M., 2000. Changes in sterol composition with ontogeny of *Blumeria graminis* conidia. Can. J. Bot. 78, 1288–1293.

Muchembled, J., Lounès-Hadj Sahraoui, A., Laruelle, F., Palhol, F., Couturier, D., Grandmougin-Ferjani, A., Sancholle, M., 2005. Methoxylated fatty acids in *Blumeria graminis* conidia. Phytochemistry 66, 793–796.

Pierpoint, W.S., 1994. Salicylic acid and its derivatives in plants: medicine metabolites and messenger molecules. Adv. Bot. Res. 20, 164–235.

Rahier, A., Benveniste, P., 1989. Mass spectral identification of phytosterols. In: Nes, W.D., Parish, E. (Eds.), Analysis of Sterols and Other Biologically Significant Steroids. Academic Press, San Diego, pp. 223–250.

Reignault, P., Cogan, A., Muchembled, J., Lounès-Hadj Sahraoui, A., Durand, R., Sancholle, M., 2001. Trehalose induces resistance to powdery mildew in wheat. New Phytol. 149, 519–529.

Ride, J.P., Barber, M.S., 1987. The effects of various treatments on induced lignification and the resistance of wheat to fungi. Physiol. Mol. Plant Pathol. 31, 349–360.

Senior, I.J., Hollomon, D.W., Loeffler, R.S.T., Baldwin, B.C., 1995. Sterol composition and resistance to DMI fungicides in *Erysiphe graminis*. Pest. Sci. 45, 57–67.

Shah, J., 2005. Lipids, lipases, and lipid-modifying. Enzymes in plant disease resistance. Annu. Rev. Phytopathol. 43, 229–260.

Vernooîj, B., Uknes, S., Ward, E., Ryals, J., 1994. Salicylic acid as a signal molecule in plant–pathogen interactions. Curr. Opin. Cell. Biol. 6, 275–279.

Weete, J.D., 1980. Lipid Biochemistry of Fungi and Other Organisms, second ed. Plenum Press, New York.

Weete, J.D., Sancholle, M., Montant, C., 1983. Effects of triazoles on fungi: II. Lipid composition of *Taphrina deformans*. Biochim. Biophys. Acta 752, 19–29.

Welch, J.W., Burligame, A.L., 1973. Very long-chain fatty acids in yeast. J. Bacteriol. 115, 464–466.