European Journal of Plant Pathology (2006) 114:405–413
DOI 10.1007/s10658-006-0005-5

Chitosan stimulates defense reactions in grapevine leaves and inhibits development of *Botrytis cinerea*

Patricia Trotel-Aziz*, Michel Couderchet, Guy Vernet and Aziz Aziz
*Laboratoire d'Eco-Toxicologie, URVVC – EA 2069, Université de Reims Champagne-Ardenne, BP 1039, 51687 Reims, cedex 2, France; Present address: Laboratoire de Plantes, Pesticides et Développement Durable, URVVC – EA 2069, Université de Reims Champagne-Ardenne, BP 1039, 51687 Reims, cedex 2, France; *Author for correspondence (E-mail: patricia.trotel-aziz@univ-reims.fr)*

Accepted 11 January 2006

Key words: chitinase, elicitor, grey mould, lipoxygenase, Phe ammonia-lyase, *Vitis vinifera*

Abstract

Chitosan (β-1,4-linked glucosamine oligomer) derived from crab shells conferred a high protection of grapevine leaves against grey mould caused by *Botrytis cinerea*. Under controlled conditions, it was shown to be an efficient elicitor of some defense reactions in grapevine leaves and to inhibit directly the *in vitro* development of *B. cinerea*. Treatment of grapevine leaves by chitosan led to marked induction of lipoxygenase (LOX), phenylalanine ammonia-lyase (PAL) and chitinase activities, three markers of plant defense responses. Dose-response curves show that maximum defense reactions (PAL and chitinase activities) and strong reduction of *B. cinerea* infection were achieved with 75–150 mg l^{-1} chitosan. However, greater concentrations of chitosan did not protect grapevine leaves with the same efficiency, but inhibited mycelial growth *in vitro*. Present results underlined the potency of chitosan in inducing some defense responses in grapevine leaves which in turn might improve resistance to grey mould.

Abbreviations: LOX – lipoxygenase; PAL – phenylalanine ammonia-lyase; PR – pathogenesis related

Introduction

Plants are able to defend themselves successfully against pathogens with a complex set of preformed structures and inducible reactions. The inducible reactions require the perception of signal molecules resulting from pathogen attack. This recognition triggers a wide range of plant defense mechanisms used for protecting against the invading pathogen. These include stimulation of the phenylpropanoid and fatty acid pathways, production of defense-specific chemical messengers such as salicylic acid (SA) or jasmonates, and accumulation of components with antimicrobial activities such as phytoalexins and pathogenesis-related (PR) proteins (Kombrink and Somssich, 1995).

Different cell wall constituents, originating from the host or even from the invading pathogen, can induce plant defense reactions (Davis and Hahlbrock, 1987; Bohland et al., 1997). These so-called elicitors are of diverse chemical nature and include proteins, peptides, glycoproteins and oligosaccharides (Côté et al., 1998; Vander et al., 1998; Rustérucci et al., 1999; Klarzynski et al., 2000). For over a decade, the possibility of stimulating plant defense responses following perception of an exogenous elicitor has become a mean for enhancing natural resistance mechanisms (Metraux et al., 1991). There is some evidence that

a number of well-defined oligosaccharides including microbial or algal β-1,3 glucans, chitin and chitosan-derived oligomers as well as the plant pectin fragments oligogalacturonides enhanced non-host plant resistance against pathogens (Roby et al., 1987; Côté et al., 1998), and it is possible that mimicking pathogen attack with such non-specific elicitors could become an alternative strategy in crop plant protection (Klarzynski et al., 2000).

Chitosan (β-1,4-linked glucosamine) is a deacylated derivative of chitin which is an important waste product from the shrimp and crab fisheries. Chitin is a component of the cell walls of many fungi (Bartnicki-Garcia, 1970), and plants produce many degrading enzymes such as chitinases, a group of PR proteins that are activated during infection and for which a direct activity against pathogen has been demonstrated (Derckel et al., 1999). Chitin may be released and converted to chitosan by deacetylation reaction, which in turn can be recognized by the plant cells (Bohland et al., 1997; Vander et al., 1998). Chitosan induces various defense responses in several plant systems, among them phytoalexin synthesis in pea pods (Hadwiger and Beckman, 1980), production of PR proteins in the leaves of rice seedlings (Agrawal et al., 2002) and lignification in wounded and intact wheat leaves (Barber et al., 1989; Vander et al., 1998), thereby inhibiting fungal growth and promoting protection from further infection (Hadwiger and Beckman, 1980). The activity of chitosan was shown to be dependent on the molecular weight (MW) and the acetylation degree (DA) of the molecule (Kauss et al., 1989; Vander et al., 1998). In melon plant, chitosan oligomers with a DA over 10% were shown to stimulate chitinase activity (Roby et al., 1987), and with a DA of less than 20% also acted as an elicitor of Phe ammonia-lyase (PAL) activity in wheat leaves (Vander et al., 1998).

The fungicidal activity of chitosan has also been documented in *in vitro* studies (El Ghaouth et al., 1992). The polycationic nature of this compound is considered a key to its antifungal properties and the length of the polymer chain enhances its antifungal activity (Hirano and Nagao, 1989). Chitosan also induces marked morphological changes and structural alterations of the fungal cells (El Ghaouth et al., 1992; Aït Barka et al., 2004).

Grapevine (*Vitis vinifera*) is susceptible to many fungal diseases, especially grey mould caused by *Botrytis cinerea*. Conidia are deposited primarily from airborne inoculum or in water on leaves, buds, canes and in bunches. Although the most prominent symptoms are found on berries, leaves constitute an early target for the fungus and contribute to the inoculum load (Galet, 1977). In addition, *B. cinerea* may reduce the plant photosynthetic surface which in turn alters the source-sink relation and wine quality. The grape producers depend mainly on chemical treatments for controlling this disease (Couderchet, 2003). However, the development of fungicide-resistant strains of *B. cinerea* has greatly reduced the effectiveness of many chemicals (Leroux et al., 1999). Finding new solutions to counteract such loss of activity of synthetic fungicides is a challenge for the future. One of the approaches is breeding for plant resistance by crossing resistant varieties with cultivated susceptible varieties. Induced plant resistance to the pathogen by external elicitors could represent another alternative method to current chemical useage which has become an environmental issue.

In response to *Botrytis* infection, grapevine leaves accumulate many defense products, including secondary metabolites and the PR proteins, chitinase and β-1,3-glucanase (Busam et al., 1997; Derckel et al., 1999), while mature berries become susceptible through the disappearance or modification of preformed defensive compounds such as proanthocyanidins and phytoalexins (Pezet et al., 1992; Jeandet et al., 1995). Thus, in the interaction of grapevine with pathogenic fungi, most reports about defense responses include expression of PR proteins and their corresponding genes (Busam et al., 1997; Derckel et al. 1999; Bézier et al., 2002), and the production of stilbene phytoalexins. Recent studies have shown that elicitors derived from plant or marine algae cell wall structures also induced various defense responses in cells and leaves of grapevine (Aziz et al., 2003, 2004).

In the above-mentioned studies, no causal link can be drawn between the elicitation of defense responses by chitosan and the establishment of resistance in grapevine. In this context, we have investigated the elicitation of defense reactions as well as the induction of resistance in grapevine leaves after application of chitosan, derived from crustaceans. Analysis of induced reactions, including stimulation of lipoxygenase (LOX), PAL, and chitinase activities was conducted. The direct effect

of chitosan on fungal growth has also been investigated. All the experiments were conducted on detached leaves which mimic within short periods of time some of the plant defense mechanisms and the common symptoms of grey mould.

Materials and methods

Plant material

Plantlets of *Vitis vinifera* cv. Chardonnay, clone 7535, were obtained *in vitro* by growing nodal explants on modified Murashige and Skoog (1962) medium (half concentration of macroelements and glutamine at 200 mg l^{-1} as nitrogen source), supplemented with 20 g l^{-1} sucrose, and 7 g l^{-1} agar. Plants were grown under white fluorescent lamps (60 μmol m^{-2} s^{-1}), 16/8 h photoperiod, and 25 °C day/night temperature. Multiplication of plants was performed in 25 mm test tubes containing 15 ml of medium per tube.

Fungal pathogen

Botrytis cinerea strain used in this study (strain 630 isolated from grapevine berries of a vineyard in the Marne Valley, France) was a gift of Dr. Y. Brygoo (INRA, Versailles, France). It was cultured in Petri dishes on potato dextrose agar (PDA, Sigma) at 22 °C for 14 days. Conidial suspension was obtained by flooding the fungal culture with sterile distilled water (SDW), rubbing the mycelium and filtering through a sterile nylon gauze (mesh of 200 μm). The conidial suspension was adjusted with SDW to 2.5×10^5 conidia ml^{-1}.

Chitosan

Chitosan originating from crustaceans with DA 10% and MW 5 KDa was purchased from AGROSOL SA (Saint-Martin l'Heureux, France).

Protection assays

Grapevine leaves excised from plantlets (10 week-old) were incubated on a 2 mM MES buffer (Sigma, pH 5.9) containing 0.5 mM $CaCl_2$ and 0.5 mM K_2SO_4 supplemented with different concentrations of chitosan (0–300 mg l^{-1}). After a 20 h period, leaves were blotted dry and placed in Petri dishes, the adaxial side facing wet absorbant paper. One needle-prick wound was applied to each leaf, and the fresh wounds were covered with 5 μl drops of the conidial suspension (2.5×10^5 conidia ml^{-1}). Disease development was measured as average diameter of lesions formed during infection of grapevine leaves with *B. cinerea*. Measurements were stopped after 7 days when the control leaves were highly infected.

Antifungal activity

In vitro assays were carried out on plates with chitosan dissolved in PDA at different concentrations (0, 75, 150, 200, 250, 300 mg l^{-1}). The PDA plates were inoculated in the centre with a 10 μl conidial suspension of *B. cinerea* and placed at 22 °C. Fungal growth was measured daily for 5 days and expressed as average diameter (mm).

Treatment of grapevine leaves

Grapevine leaves detached from 10 week-old plantlets were incubated in the culture chamber on the MES buffer containing different concentrations of chitosan (0–300 mg l^{-1}). At different times after treatment, leaves were blotted dry, fixed in liquid nitrogen, and stored at −80 °C before analysis.

LOX activity

The frozen leaves (250 mg fresh weight, FW) were ground with pestle and mortar at 4 °C with polyvinylpolypyrrolidone (PVPP, Sigma, 1% w/w) and 1 ml of sodium phosphate buffer (50 mM, pH 6.5) containing Triton X-100 (0.25% v/v) and phenylmethylsulfonyl fluoride (PMSF, Sigma, 1 mM). The homogenate was centrifuged at 20,000*g* for 30 min (4 °C). The supernatant was kept on ice and used as enzymatic extract.

LOX activity was determined according to the method of Axelrod et al. (1981) using linoleic acid as a substrate. Enzyme assays were prepared by addition of enzyme extract (25 μl) to a mixture containing 100 μl of linoleic acid (10 mM) and 0.875 ml of phosphate buffer (50 mM, pH 6.0). The reaction was conducted at 25 °C and LOX activity was determined by the increase in absorbance at 234 nm during 1, 2.5 and 5 min. The same procedure was used for the blank reaction in

which phosphate buffer (pH 6.5) replaced leaf extract. The hydroperoxides formed during enzymatic reaction were quantified by using a molar extinction coefficient of 25,000 M^{-1} cm^{-1}.

PAL activity

The enzyme was extracted at 4 °C from frozen leaves (250 mg FW) ground with PVPP (1% w/w) and 1 ml of potassium phosphate buffer (100 mM, pH 8.0) containing β-mercaptoethanol (1.4 mM). The homogenate was centrifuged at 15,000*g* for 15 min (4 °C), and the resulting supernatant was used as the crude enzyme extract.

PAL activity was determined according to the method of Tanaka et al. (1974) slightly modified. The reaction mixture was prepared by adding 0.45 ml of Tris–HCl buffer (100 mM, pH 8.8) and 0.2 ml of phenylalanine (40 mM) to 0.15 ml of enzyme extract. The mixture was incubated for 10, 20 and 30 min at 37 °C and reaction was stopped by adding 0.2 ml trichloroacetic acid (TCA, 25%). The assay mixture was centrifuged at 10,000*g* for 15 min (4 °C) and absorbance of the supernatant was measured at 280 nm. A molar extinction coefficient of 17.4 mM^{-1} cm^{-1} was used to quantify cinnamic acids formed during the enzymatic reaction.

Chitinase activity

The chitinases were extracted at 4 °C from frozen leaves (250 mg FW) ground with PVPP (1% w/w) and sodium acetate buffer (50 mM, pH 5.0) containing DTT (1 mM) and PMSF (0.2% w/v). The mixture was then centrifuged at 9000*g* for 5 min (4 °C). The resulting supernatant containing the crude extract was further diluted by 1/10 for the chitinase assay.

Chitinase activity was determined according to the method described by Byrne et al. (2001) using CarboxyMethyl-chitin-Remazol Brillant Violet 5R (CM-chitin-RBV, LOEWE Biochemica, Germany) as a substrate. The diluted extract (50 μl) was incubated at 37 °C in an Eppendorf microtube containing 0.1 ml of CM-chitin-RVB, 0.15 ml of distilled water and 0.1 ml of sodium acetate buffer (200 mM, pH 5.0) for 0, 1, 2.5 and 5 min. Enzymatic reaction was stopped by the addition of 0.4 ml HCl (0.3 M). The microtubes were then cooled on ice for 10 min before being centrifuged at 9000*g* for 10 min (4 °C). The absorbance of supernatant was measured at 550 nm.

Results

Chitosan-induced protection of grapevine leaves against Botrytis cinerea

The spreading of necrotic lesions caused by *B. cinerea* (Table 1) was strongly reduced in the presence of 75 mg l^{-1} chitosan as compared to the control (incubated on buffer alone) that showed severe disease symptoms. With 150 mg l^{-1} chitosan, the lesion was entirely suppressed indicating a complete protective effect on plantlet leaves post-infected with *B. cinerea*. Nevertheless, when chitosan concentration exceeded 200 mg l^{-1}, the leaf tissues appeared damaged as shown by an increase in the spread of necrotic lesions.

Chitosan antifungal activity

The growth of *B. cinerea* was inhibited by chitosan in a dose-dependent manner (Figure 1). A reduction of *B. cinerea* growth started with 75 mg l^{-1} of chitosan. With 150 mg l^{-1} chitosan, the fungal growth decreased by 65% and was almost entirely suppressed with a 250 mg l^{-1} solution. These results indicate a strong antagonistic effect of chitosan on pathogen growth.

Table 1. Protective effect of chitosan on plantlet leaves post infected with *Botrytis cinerea*

Chitosan (mg l^{-1})	Necrotic lesion (mm)	Corresponding Protection (%)
0	20 ± 3	0 ± 0
75	6 ± 2	70 ± 10
150	0 ± 0	100 ± 0
200	2.4 ± 2.2	88 ± 11
250	4.6 ± 1.8	77 ± 9
300	10 ± 2	50 ± 10

Grape leaves were floated for 20 h on a buffer solution containing different concentrations of chitosan (0–300 mg l^{-1}), removed from the solution, and infected with a conidial suspension of *Botrytis*. Necrotic lesions were measured 7 days after infection. Each treatment was performed in triplicate with at least 10 leaves in each assay. Data are means of 2 independent experiments.

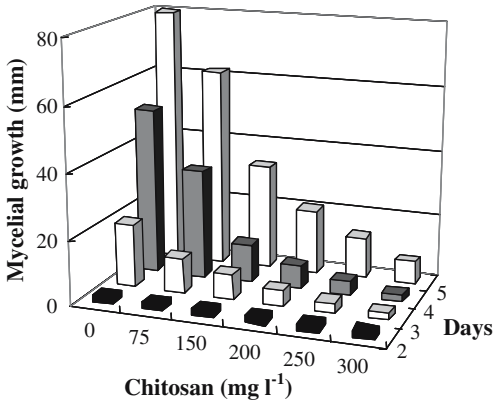

Figure 1. Effect of chitosan on the radial growth of *Botrytis cinerea* on agar plates containing different concentrations of chitosan (0, 75, 150, 200, 250, 300 mg l^{-1}). Data are means of 2 independent experiments with 3 replicates.

The effect of chitosan on LOX, PAL and chitinase activities

Defense responses of grapevine leaves subjected or not to chitosan were examined by the measurement of lipoxygenase (LOX), phenylalanine ammonia-lyase (PAL) and chitinase activities. LOX activity was transiently increased in excised grapevine leaves incubated with chitosan (Figure 2). This increase occurred earlier with higher concentrations of chitosan; it was different from the control after 2 h with 150 mg l^{-1}. Also, the maximum activity was observed after 6 h for 75 mg l^{-1} and after 4 h for high concentrations. Furthermore, LOX activity increased with increasing concentration of chitosan. Compared to the control, LOX activity in grapevine leaves almost doubled after 4 h of incubation with 300 mg l^{-1} of chitosan.

PAL activity also transiently increased in excised grapevine leaves incubated with chitosan (Figure 3). This activity appeared biphasic with a short early peak after 2–4 h which preceded a longer increase in PAL activity after 8 h of incubation. The first peak was approximately five times higher after 2 h in the presence of the lowest concentrations of chitosan (75 and 150 mg l^{-1}) and up to ten times higher after 4 h in the presence of 250 mg l^{-1} of chitosan. However, the amplitude of this first peak decreased when chitosan concentration reached 300 mg l^{-1}. The second phase of PAL activity was also dependent on the concentration of chitosan. PAL activity reached maximum values with low concentrations of chitosan (75 and 150 mg l^{-1}). Furthermore, this maximum was reached earlier when grapevine

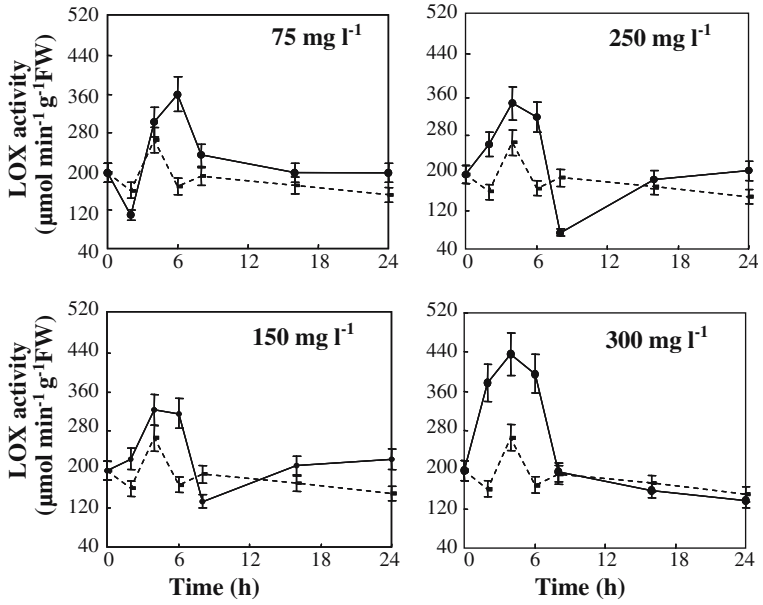

Figure 2. LOX activity in grapevine leaves incubated in the standard buffer (dashed line) supplemented with chitosan (solid line) at different concentrations (75–300 mg l^{-1}). Data ± SE are means of 2 independent experiments with 3 replicates.

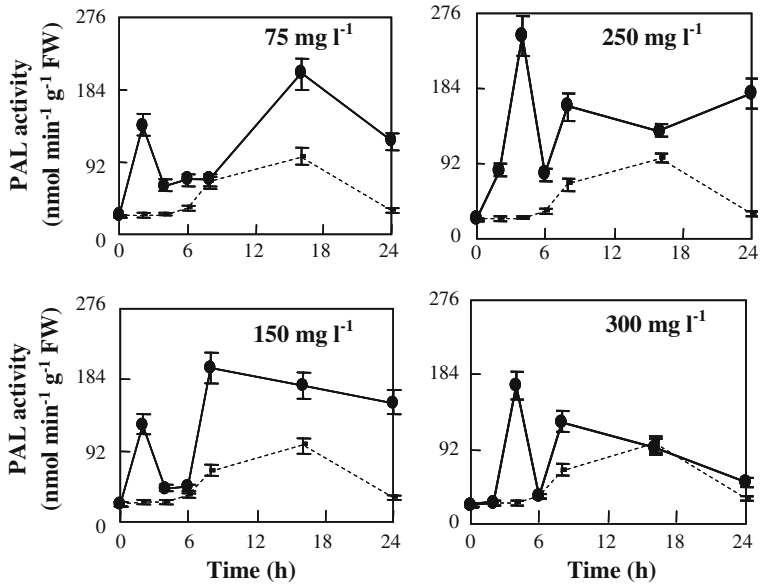

Figure 3. PAL activity in grapevine leaves incubated in the standard buffer (dashed line) supplemented with chitosan (solid line) at different concentrations (75–300 mg l^{-1}). Data ± SE are means of 2 independent experiments with 3 replicates.

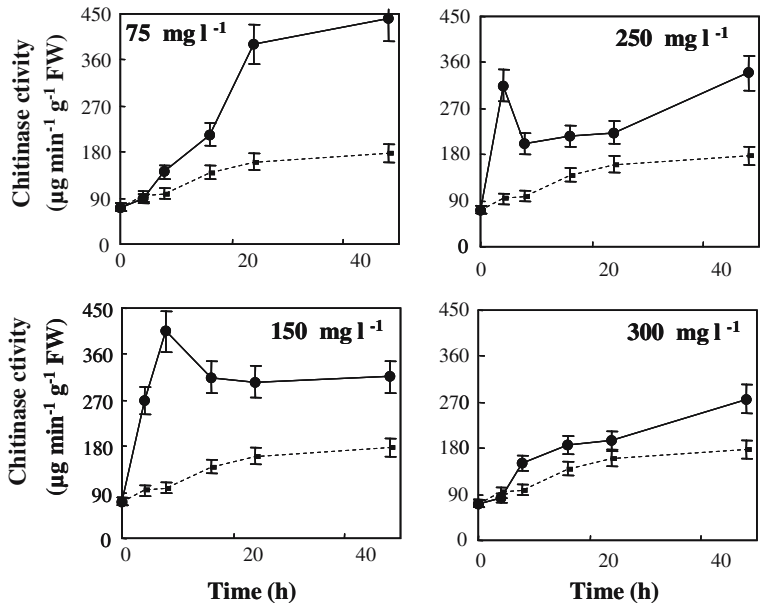

Figure 4. Chitinase activity in grapevine leaves incubated in the standard buffer (dashed line) supplemented with chitosan (solid line) at different concentrations (75–300 mg l^{-1}). Data ± SE are means of 2 independent experiments with 3 replicates.

leaves were incubated with 150 mg l^{-1} of chitosan (after 8 h) than with 75 mg l^{-1} (after 16 h). In the presence of chitosan at concentrations exceeding 150 mg l^{-1}, the increase of PAL activity was less.

Chitinase activity also increased in response to chitosan applications (Figure 4). It reached its maximum value with low concentrations of chitosan (75 and 150 mg l^{-1}). The maximum was reached earlier when leaves were incubated with 150 mg l^{-1} of chitosan (after 8 h) than with 75 mg l^{-1} (after 24 h). Above 150 mg l^{-1}, chitinase activity increase was less.

Discussion

The data presented in this paper show that chitosan confers a good level of protection for grapevine leaves against *B. cinerea*. Chitosan can act through induction of plant defense reactions or directly by affecting the fungal growth. The spreading of necrotic lesions caused by *B. cinerea* was significantly reduced when grapevine leaves were incubated with chitosan before inoculation. Treatment with 150 mg l^{-1} chitosan was more effective than higher concentrations. Such efficacy of these low concentrations suggests that this compound was recognized by plant cells and the observed protection resulted at least in part from the induction of plant defense responses. The lower efficacy of chitosan above 150 mg l^{-1} remains a matter for debate.

Following chitosan treatment of grapevine leaves, three enzymatic defense markers were investigated. LOX activity, a putative control point for fatty acid-derived signalling pathways (Blée, 1998), was stimulated after 2 h of incubation with chitosan, with the maximum induction reached between 4 and 6 h after treatment (Figure 2). Within a few hours chitosan also activated the phenylpropanoid pathways, as shown by the marked increase in PAL activity, with a maximum reached 4 h after the addition of the elicitor. LOX is required for the synthesis of the precursors of jasmonates, compounds that may act as the signal factor in plant defense responses (Creelman and Mullet, 1997). PAL is a key enzyme concerned with the synthesis of secondary metabolites, especially the production of phytoalexins and salicylic acid (SA) (Lee et al., 1995; Shadle et al., 2003), which were proposed to reduce incidence of plant disease through antifungal activity and stimulation of plant defense responses, respectively (Reymond and Farmer, 1998; Jeandet et al., 2002). The enhancement of these two activities was already reported for interactions of chitosan and wheat (Bohland et al., 1997; Vander et al., 1998), and β-glucans and tobacco (Klarzynski et al., 2000). It is interesting to note that PAL activity was biphasic in response to chitosan and that the second phase was higher at the most effective concentration of chitosan. Similar results have been shown in melon cotyledons treated with cellulase derived from *Trichoderma* spp. (Martinez et al., 2001). These authors suggested that the early PAL activation could be required for SA biosynthesis, which in turn caused an increase in PAL activity (second peak), thereby reinforcing the grapevine defense responses.

Chitinase activity was also increased as early as 2 h after treatment with chitosan in grapevine leaves and was maintained for at least 25 h. It should be noted that chitinase is induced best by the most effective concentration in the protection test. Chitinase is one of the PR proteins that have well-described antimicrobial activities against different pathogens. Based on their hydrolytic activities (Van Loon and Van Strien, 1999), chitinases might also play an important role in the amplification of defense reactions through release of chitin from the pathogen cell walls (Kurosaki et al., 1988).

In the present dose-response experiments with chitosan, LOX activity was not tightly correlated with the increases in PAL and chitinase activities. Although PAL and chitinase activities were strongly stimulated at chitosan doses as low as 75 to 150 mg l^{-1}, higher induction of LOX activity was achieved by concentrations exceeding 150 mg l^{-1}. Taking into account that high concentrations of chitosan resulted in a reduction of resistance efficiency to *B. cinerea*, it is suggested that higher LOX activity could be related to a possible induced-lipid peroxidation and membrane damages as reported earlier during the hypersensitive reaction (Rustérucci et al., 1999). Thus, the low level of protection is in accordance with the decline of PAL and chitinase activities above 150 mg l^{-1} chitosan. This indicates that biological protection of grapevine leaves against *B. cinerea* and activation of plant resistance are both dependent on chitosan concentration.

Our studies confirm other findings on direct antifungal activity of chitosan *in vitro* (Allan and Hadwiger, 1979; Aït Barka et al., 2004) and show that the level of mycelial growth inhibition is highly correlated with chitosan concentration. It has been reported that the polycationic nature of chitosan is considered to be a key to its antifungal properties (Hirano and Nagao, 1989). However, in the present work, low concentrations of chitosan on PDA medium only partly inhibited *B. cinerea* growth while it was strongly inhibitory on leaves at the same concentrations; therefore because it activated various reactions in grapevine leaves, it is likely that the chitosan-induced resistance to

B. cinerea strongly results from the stimulation of the plant's natural defense metabolism. The fact that grapevine leaves were washed before challenging the leaves with *B. cinerea* is another factor in favour of the induction of defense responses by chitosan as part of the inhibition of pathogen progression in host tissues.

Our data provide evidence that chitosan at low concentrations is a potent inducer of defense reactions in grapevine leaves and a direct inhibitor of *B. cinerea* growth under *in vitro* conditions, both mechanisms improving resistance to grey mould. Current evidence therefore suggests this natural substance appears to control grey mould on leaves. Further experiments are in progress on grape berries to provide evidence that chitosan is a good alternative to synthetic fungicides in the vineyards.

Acknowledgements

This work was supported by Europol'Agro through the program VINEAL. Special thanks are due to Professor C. Clément (Laboratoire de Stress, Défense et Reproduction des Plantes) for allowing PTA to use laboratory facilities.

References

Agrawal GK, Rakwal R, Tamogami S, Yonekura M, Kubo A and Saji H (2002) Chitosan activates defense/stress response(s) in the leaves of *Oryza sativa* seedlings. Plant Physiology and Biochemistry 40: 1061–1069.

Aït Barka E, Eullaffroy P, Clément C and Vernet G (2004) Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. Plant Cell Reports 22: 608–614.

Allan CR and Hadwiger LA (1979) The fungicidal effect of chitosan on fungi of varying cell wall composition. Experimental Mycology 3: 285–287.

Axelrod B, Cheesbrough TM and Laakso S (1981) Lipoxygenases from soybeans. In: Lowenstein JM (ed.) Methods in Enzymology, Vol. 71 (pp. 441–451) Academic Press, New York.

Aziz A, Poinssot B, Daire X, Adrian M, Bézier A, Lambert B, Joubert JM and Pugin A (2003) Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. Molecular Plant Microbe Interaction 16: 1118–1128.

Aziz A, Heyraud A and Lambert B (2004) Oligogalacturonide signal transduction, induction of defense-related responses and protection of grapevine against *Botrytis cinerea*. Planta 218: 767–774.

Barber MS, Bertram RE and Ride JP (1989) Chitin oligosaccharides elicit lignification in wounded leaves. Physiological and Molecular Plant Pathology 34: 3–12.

Bartnicki-Garcia S (1970) Cell wall chemistry, morphogenesis, and taxonomy of fungi. Annual Review of Microbiology 22: 87–108.

Bézier A, Lambert B and Baillieul F (2002) Study of defense-related gene expression in grapevine leaves and berries infected with *Botrytis cinerea*. European Journal of Plant Pathology 108: 111–120.

Blée E (1998) Phytooxylipins and plant defense reactions. Progress in Lipid Research 37: 33–72.

Bohland C, Balkenhohl T, Loers G, Feussner I and Grambow HJ (1997) Differential induction of lipoxygenase isoforms in wheat upon treatment with rust fungus elicitor, chitin oligosaccharides, chitosan, and methyl jasmonate. Plant Physiology 114: 679–685.

Busam G, Kassemeyer HH and Matern U (1997) Differential expression of chitinases in *Vitis vinifera* L. responding to systemic acquired resistance activators or fungal challenge. Plant Physiology 115: 1029–1038.

Byrne ND, Duxbury M and Sharpe N (2001) The determination of chitinase activity of grapes: an introductory enzyme assay. Biochemistry and Molecular Biology Education 29: 144–146.

Côté F, Laflamme L, Payet MD and Gallot-Payet N (1998) Oligosaccharide elicitors in host-pathogen interactions. Generation, perception, and signal transduction. Subcellular Biochemitry 29: 385–432.

Couderchet M (2003) Benefits and problems of fungicide control of *Botrytis cinerea* in vineyards of Champagne. Vitis 42: 165–171.

Creelman RA and Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annual Review of Plant Physiology and Plant Molecular Biology 48: 355–381.

Davis KR and Hahlbrock K (1987) Induction of defense responses in culture parsley cells by plant cell wall fragments. Plant Physiology 84: 1286–1290.

Derckel JP, Baillieul F, Manteau S, Audran JC, Haye B, Lambert B and Legendre L (1999) Differential induction of grapevine defenses by two strains of *Botrytis cinerea*. Phytopathology 89: 197–203.

El Ghaouth A, Arul J, Grenier J and Asselin A (1992) Antifungal activity of chitosan on two post-harvest pathogens of strawberry fruits. Phytopathology 82: 398–402.

Galet P (1977) Les maladies cryptogamiques: Pourriture grise. In: Galet P (ed.) Les maladies et les parasites de la vigne (pp. 313–360) Tome I. Editions Lavoisier, France.

Hadwiger LA and Beckman JM (1980) Chitosan as a component of pea-*Fusarium solani* interactions. Plant Physiology 66: 205–211.

Hirano S and Nagao N (1989) Effects of chitosan, pectic acid, lysozyme, and chitinase on the growth of several phytopathogens. Agricultural and Biological Chemistry 53: 3065–3066.

Jeandet P, Bessis R, Sbaghi M and Meunier P (1995) Production of the phytoalexin resveratrol by grapes as a response to *Botrytis* attack under natural conditions. Journal of Phytopathology 143: 135–139.

Jeandet P, Douillet-Breuil AC, Bessis R, Debord S, Sbaghi M and Adrian M (2002) Phytoalexins from the *Vitaceae*:

biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity and metabolism. Journal of Agricultural and Food Chemistry 50: 2731–2741.

Kauss H, Jeblick W and Domard A (1989) The degrees of polymerization and N-acetylation of chitosan determine its ability to elicit callose formation in suspension cells and protoplasts of *Catharanthus roseus*. Planta 178: 385–392.

Klarzynski O, Plesse B, Joubert JM, Yvin JC, Kopp M, Kloareg B and Fritig B (2000) Linear β−1,3-glucans are elicitors of defense responses in tobacco. Plant Physiology 124: 1027–1037.

Kombrink E and Somssich IE (1995) Defense responses of plants to pathogens. Advances in Botanical Research 21: 1–34.

Kurosaki F, Tashiro N and Nishi A (1988) Role of chitinase and chitin oligosaccharides in lignification response of cultured carrot cells treated with mycelial walls. Plant and Cell Physiology 29: 527–531.

Lee H, Leon J and Raskin I (1995) Biosynthesis and metabolism of salicylic acid. Proceedings of the National Academy of Sciences of the United States of America 92: 4076–4079.

Leroux P, Chapeland F, Desbrosses D and Gredt M (1999) Patterns of cross-resistance to fungicides in *Botryotinia fuckeliana* (*Botrytis cinerea*) isolates from French vineyards. Crop Protection 18: 687–697.

Martinez C, Blanc F, Le Claire E, Besnard O, Nicole M and Baccou JC (2001) Salicylic acid and ethylene pathways are differentially activated in melon cotyledons by active or heat-denaturated cellulase from *Trichoderma longibrachiatum*. Plant Physiology 127: 334–344.

Metraux JP, Ahl-Goy P, Staub T, Speich J, Steinemann A, Ryals J and Ward ER (1991) Induced resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens. In: Hennecke H and Verma DPS (eds.) Advances in Molecular Genetics of Plant Microbe Interactions, Vol. I (pp. 432–439) Kluwer Academic Press, the Netherlands.

Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco cultures. Physiologia Plantarum 15: 473–496.

Pezet R, Pont V and Hoang-Van K (1992) Enzymatic detoxication of stilbenes by *Botrytis cinerea* and inhibition by grape berries proanthocyanidins. In: Verhoeff K, Malathrakis NE and Williamson B (eds.) Recent advances in *Botrytis* research. Proceedings of the 10th international *Botrytis* Symposium (pp. 87–92) Pudoc Scientific Publishers, Washington, USA.

Reymond P and Farmer EE (1998) Jasmonate and salicylate as global signals for defense gene expression. Current Opinion in Plant Biology 1: 404–411.

Roby D, Gadelle A and Toppan A (1987) Chitin oligosaccharides as elicitors of chitinase activity in melon plants. Biochemical and Biophysical Research Communications 143: 885–892.

Rustérucci C, Montillet JL, Agnel JP, Battesti C, Alonso B, Knoll A, Bessoule JJ, Etienne P, Stuty L, Blein JP and Triantaphylides C (1999) Involvement of lipoxygenase-dependent production of fatty acid hydroperoxides in the development of the hypersensitive cell death induced by cryptogein of tobacco leaves. Journal of Biological chemistry 274: 36446–36455.

Shadle GL, Wesley V, Korth KL, Chen F, Lamb C and Dixon RA (2003) Phenylpropanoid compounds and disease resistance in transgenic tobacco with altered expression of L-phenylalanine ammonia-lyase. Phytochemistry 64: 153–161.

Tanaka Y, Kojima M and Uritani I (1974) Properties, development and cellular-localization of cinnamic acid 4-hydroxylase in cut-injured sweet potato. Plant and Cell Physiology 15: 843–854.

Van Loon LC and Van Strien EA (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiological and Molecular Plant Pathology 55: 85–97.

Vander P, Kjell MV, Domard A, El-Gueddari NE and Moerschbacher BM (1998) Comparison of the ability of partially N-acetylated chitosans and oligosaccharides to elicit resistance in wheat leaves. Plant Physiology 118: 1353–1359.