Differential Induction of Grapevine Defenses by Two Strains of *Botrytis cinerea*

Jean-Paul Derckel, Fabienne Baillieul, Sébastien Manteau, Jean-Claude Audran,
Bernard Haye, Bernard Lambert, and Laurent Legendre

First, second, sixth, and seventh authors: Laboratoire de Biologie et Physiologie Végétales, Equipe de Biochimie et Biologie Moléculaire des Plantes, UPRES-EA 2069; third author: Laboratoire d'Oenologie, UPRES-EA 2069; fourth author: Laboratoire de Biologie et Physiologie Végétales, UPRES-EA 2069; and fifth author: Laboratoire de Biochimie; at: Université de Reims Champagne-Ardenne, UFR Sciences, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France.

Accepted for publication 20 November 1998.

ABSTRACT

Derckel, J.-P., Baillieul, F., Manteau, S., Audran, J.-C., Haye, B., Lambert, B., and Legendre, L. 1999. Differential induction of grapevine defenses by two strains of *Botrytis cinerea*. *Phytopathology* 89:197-203.

Even though *Botrytis cinerea*, the causal agent of gray mold, is a highly variable fungus with strains displaying very different degrees of virulence toward one given host plant species, no study has yet shown any correlation between the lack of aggressiveness of one given strain and its ability to stimulate a defense response from its host. Strains of *B. cinerea* collected from different host plant species were screened for their pathogenicity on grapevine to select two strains with similar morphological characteristics but different levels of virulence. In grapevine

leaves, the less aggressive strain, T4, enhanced the accumulation of many defense products including secondary metabolites and the pathogenesis-related proteins, chitinase and β -1,3-glucanase. Interestingly, secondary metabolites were formed in cells around a small group of dead cells. When compared with T4, the more aggressive strain, T8, had larger necrotic spots, no secondary metabolite biosynthesis, and accumulations of chitinases and β -1,3-glucanases that were more delayed, yet only slightly weaker. The culture fluids of both strains mimicked the differential effect of each isolate in stimulating chitinase activity when infiltrated into grapevine leaves.

Additional keywords: hypersensitive-like response, plant defenses, secondary metabolism, *Vitis vinifera*.

Among fungal plant pathogens, *Botrytis cinerea* Pers.:Fr. displays one of the widest host range specificity. This causal agent of gray mold infects a large range of plant genera as well as plant tissues. These include the fruits tomato, strawberry, and raspberry and the flowers tulip, rose, and orchid (4). The grapevine industry is particularly affected by this fungus that attacks grapes at a pre- and postharvest stage under a wide array of environmental conditions and on a large geographical area. Even though less damaging than fruit loss, this fungus may also develop on the leaves and the woody stems of grapevine plants.

Numerous studies have revealed that *B. cinerea* is a highly variable fungal species. Early work (36) showed that *B. cinerea* isolates differed morphologically and physiologically. This observation was confirmed later (16,19,31). Strains of *B. cinerea* isolated from different host plants differ in secretion or accumulation of enzymes such as pectin methyl esterase (46), polygalacturonase (10), phenol oxidase (46), acid proteases (47), hexose oxidase (12), and β -glucosidase (41). Moreover, a genetic analysis of a large population of *B. cinerea* additionally revealed large differences among members such that some authors suggested the *B. cinerea* complex could be subdivided into two subspecies (14). More importantly, various *B. cinerea* strains have been shown to differ in their aggressiveness toward one given host plant species (4,10,47).

Plants resist pathogen infection through physical and chemical defenses that may be either preformed (cuticle and cell wall) or induced after pathogen penetration. Induced defenses appear at different times after pathogen recognition and at varying distances from the infected area. They include, in chronological order, pro-

duction of oxidative species, cell wall strengthening, phytoalexin biosynthesis, and the accumulation of defense-related proteins such as pathogenesis-related proteins (26). Interestingly, good correlations have been found between the presence in certain plants of some of these defense compounds and their higher tolerance toward *Botrytis*. The grapevine phytoalexin, resveratrol, was shown to control *B. cinerea* growth both in vitro (20) and in planta after its artificial accumulation in transgenic tobacco plants expressing the grapevine stilbene synthase gene (18). In addition, a strong correlation has been found between the ability of grapevine cultivars to synthesize resveratrol in response to UV irradiation and their tolerance toward *B. cinerea* (21). The increased production of the French bean phytoalexin, phaseollin, during an incompatible interaction with a particular *B. cinerea* strain has, however, been considered (45) to be the result and not the determining factor of the increased resistance of primary French bean leaves. Cell wall lignification (15) as well as callose and suberin deposition (5) have additionally been suggested to account for the restriction of the pathogen's mycelium in young tomato fruits. Increased papillae formation has been seen in broad bean and tulip leaves during their incompatible interaction with nonpathogenic *Botrytis* species (32). Finally, the artificial expression of the defense-related proteins, chitinase, and β -1,3-glucanase, either alone (38) or in combination (1), has led to lower rates of lesion development. However, none of the above studies have established any correlation between the differences in aggressiveness of various *B. cinerea* isolates and their ability to stimulate the production of active defenses by their host.

In the current study, we developed a pathogenicity assay on in vitro-cultivated grapevine plants that discriminates among *B. cinerea* isolates. We selected two *B. cinerea* strains with similar physiological characteristics, but different degrees of aggressiveness toward grapevine, to estimate their respective ability to stimulate the plant defense machinery. Several defense reactions such as the

Corresponding author: L. Legendre; E-mail address: laurent.legendre@univ-reims.fr

production of phenolics and the biosynthesis of chitinases and glucanases were examined. We then evaluated whether culture filtrates of these two strains mimicked the effect of the living strains when infiltrated into grapevine leaves.

MATERIALS AND METHODS

Fungal and plant cultures. The *B. cinerea* isolates used in this study were a generous gift of Y. Brygoo (INRA, France). They were maintained on potato dextrose agar at 21°C with a 16/8-h photoperiod. Liquid cultures of the fungal strains were obtained by placing 2 × 10⁶ conidia into 150 ml of the synthetic medium (35) containing 0.1% (wt/vol) polygalacturonic acid (Sigma Chemical Co., St. Louis) and 20 g of glucose per liter. They were shaken at 160 rpm at 21°C with a 16/8-h photoperiod under cool white neon tubes (3,000 lx).

Grapevine plants (*Vitis vinifera* L. cv. Chardonnay 7535) were micropropagated in vitro at 25°C with a 16/8-h photoperiod (33). Six-week-old plantlets were used in all experiments.

Phytopathogenicity assay. A suspension of conidia was made in distilled water containing 20 g of glucose per liter from 14- to 18-day-old cultures of *B. cinerea*. The conidia concentration was adjusted to 1 × 10⁵ conidia per ml with glucose (20 g liter⁻¹) containing water before placing 10 µl of the diluted conidia suspension on each leaf of an in vitro-cultivated grapevine plant. The plants were left unharmed in their growth tubes during inoculation and were placed back in their growth chamber after inoculation. At the indicated time, infected plantlets were taken out of their growth tube to take pictures of their leaves. Each assay was conducted several times on three separate plants. On a routine basis, the extent of leaf necrosis appearing 3 days after inoculation was used as a mean of grading the aggressiveness of *B. cinerea* isolates.

Light and fluorescence microscopy. Individual grapevine leaves inoculated with the various *B. cinerea* isolates 48 h beforehand were placed without any specific treatment under the white or UV light (280 nm) of a microscope (×125). In vitro-cultivated plants were sufficiently thin to enable individual cells to be seen.

Fungus staining. Fungal hyphae were stained with fuchsin acid. The inoculated leaves were fixed during 24 h in a glacial acetic acid/ethanol (2:1) solution. Chlorophylls were removed in a lactophenol solution (40 g of phenol, 20 ml of lactic acid, and 40 ml of glycerol) for 24 h, before being immersed for 45 to 50 min in lactophenol containing 1% (wt/vol) fuchsin acid (Aldrich Chemical Co., Milwaukee, WI). After a brief washing in lactophenol, the leaves were analyzed by phase-contrast microscopy (×125 and ×600) in a solution containing 90 ml of glycerol, 9 ml of water, and 1 ml of Tween 20.

Evans blue staining. Leaves inoculated for 48 h with various *B. cinerea* isolates were stained with Evans blue after first vacuum-infiltrating distilled water. The infiltrated leaves were soaked in a 0.25% (wt/vol) Evans blue solution for 1 h before being rinsed with distilled water and photographed under a phase-contrast microscope (×125).

Chitinase and β-1,3-glucanase extraction and quantitation. Grapevine chitinases and β-1,3-glucanases were extracted by grind-

ing 230 mg of fresh leaves into 2 ml of a 50 mM sodium acetate buffer, pH 5.0, containing 1 mM dithiothreitol and 0.2% (wt/vol) phenylmethylsulfonyl fluoride. This crude extract was centrifuged at 3,000 × g for 10 min and the supernatant either used directly or precipitated with 90% (wt/vol) ammonium sulfate. The pelleted proteins were dissolved in 2 ml of 50 mM sodium acetate buffer, pH 5.0, and used to assay glucanase activity.

Chitinolytic activity was assayed with chitin-azur and acidic chitinase isoforms separated on a 7.5 to 15% native gradient gel and visualized using glycol-chitin overlays as described previously (9).

β-1,3-Glucanase activity was measured according to published procedures (11) with the following modifications. Reactions were started by mixing 25 µl of extract with 225 µl of laminarin at 0.5 mg/ml dissolved in 50 mM sodium acetate buffer, pH 5.0. The reaction was incubated at 37°C for the desired time and stopped by the addition of 500 µl of a solution containing 16 g of glycine per liter, 450 mg of CuSO₄·5H₂O per liter, and 500 µl of 1.2 mg of neocuproine-HCl per ml before boiling the whole mixture for 3 min. The quantity of reducing sugars released from laminarin was proportional to the optical density of the cooled solution at 450 nm. Glucose was used as a standard. Controls were made by using 50 mM sodium acetate buffer, pH 5.0, instead of the crude protein extracts.

Fig. 1. Phytopathogenicity assay of two *Botrytis cinerea* isolates on grapevine plants. Conidial suspensions of the *B. cinerea* isolates T4 and T8 were deposited on intact in vitro-cultivated grapevine plants and left to incubate in the plant growth chamber. At different times after inoculation, indicated on the left, individual leaves were detached and photographed. The pictures were obtained on one typical experiment, but similar results have been seen on at least three other separate assays.

TABLE 1. Morphological characteristics and virulence properties of various *Botrytis cinerea* isolates

Isolate	Original host	Germination rate (%) ^a	Hyphae branching ^{a,b}	Aggressiveness on grapevine ^b
630	Grapevine	50	—	++
616	Grapevine	40	+	++
425	Pea	100	+	+
P1	Pea	50	+	—
T4	Tomato	100	—	—
T8	Tomato	100	—	++

^a Germination rate and hyphae branching capacity were estimated after 24 h of culture in a liquid synthetic medium.

^b — = very low; — = low; + = high; and ++ = very high.

Polygalacturonase, laccase, and protease activity measurements. Polygalacturonase, laccase, and protease activity were estimated on the supernatant of 11-day-old liquid cultures of the desired *B. cinerea* isolate.

Polygalacturonase activity was determined by measuring the increase in reducing groups released from sodium polypectate with respect to time. Assays were performed at 30°C in a mixture containing 3 µg of total protein and 0.8% (wt/vol) sodium polypectate (Sigma Chemical Co.) in 50 mM sodium acetate buffer, pH 5.0. Reducing groups were measured using *p*-hydroxybenzoic acid hydrazide (Aldrich Chemical Co.) as a chromophore (29). One unit of activity was defined as 1 µmol of reducing group generated per min at 30°C in the above conditions using galacturonic acid (Sigma Chemical Co.) as a standard.

Protease activity was estimated via the degradation of acid-denatured hemoglobin (44). Degradation products were quantitated with fluorescamine. Basically, 50 µl of acid-denatured hemoglobin (Sigma Chemical Co.) was mixed at 30°C with 50 µl of 100 mM sodium citrate, pH 3.1, and 50 µl of extract. At different times, the reaction was stopped by the addition of 350 µl of ice-cold 5% trichloroacetic acid. A total of 120 µl of the supernatant was then mixed with 88 µl of 500 mM sodium carbonate, pH 9.5, and 50 µl of fluorescamine (1 mg per ml of dimethylsulfoxide) before reading the fluorescence at $\lambda_{EX} = 405$ nm and $\lambda_{EM} = 473$ nm. One unit of activity was defined as the amount of pepsin capable of generating the same amount of amine groups during the initial stages of the reaction.

Laccase activity was quantitated according to the method described by Grassin and Dubourdieu (17).

Fig. 2. Necrotic lesion formation and accumulation of phenolics in grapevine leaves inoculated with two strains of *Botrytis cinerea*. Conidial suspensions of the *B. cinerea* isolates T4 and T8 were deposited on intact in vitro-cultivated grapevine plants and left to incubate in the plant growth chamber. At 48 h after inoculation, grapevine leaves were either **A**, stained with Evans blue; or **B**, left intact and illuminated with UV light. **B**, An arrow points to the black dead cells surrounded by blue fluorescent cells. These pictures correspond to one typical experiment, but similar results have been obtained on two other separate assays.

Protein content measurement. Protein concentrations were estimated with Amidoblack (Aldrich, St. Quentin, France) according to the methods described by Schaffner and Weissman (42).

RESULTS

Comparison of the aggressiveness of various *B. cinerea* isolates on grapevine plants. To obtain two strains of *B. cinerea* exhibiting different degrees of aggressiveness toward grapevine, we surveyed several strains of *B. cinerea* isolated on different plant species including rose, tomato, and grapevine. Conidia from each isolate were inoculated on grapevine plants (Table 1). Between 2 to 10 h later, conidia started to germinate on the leaves of intact in vitro-cultivated plants. Most germ tubes elongated toward the leaf veins, and penetration of the epidermal cell layer occurred without the formation of a large appressorium, either directly through epidermal cells or at the junction between two cells (in agreement with observations made by others [13]). In very few cases, the conidia

germ tubes entered through the stomata (obtained with fuchsin acid staining of the hyphae) (data not shown). The most aggressive strains started to generate visible necrotic lesions 3 days after inoculation (Fig. 1). Lesions were brown, started from the tip of the leaf, and spread quickly through the entire leaf. New conidia were produced only 2 days later. Other isolates failed by day 3 to generate obvious visible symptoms (Table 1). A careful visual observation of the leaf lamina, however, revealed the presence of very tiny black dots (less than 0.1 mm). During all assays, growth of the fungus was evident on the plant nutritive medium about 5 to 6 days after inoculation. This did not affect the test results, which were scored on a routine basis 3 days after inoculation.

Two isolates of *B. cinerea* originating from field-grown grapevine plants were highly virulent (Table 1), while the isolates collected on other plant species exhibited low to high levels of aggressiveness toward grapevine.

All of the above isolates exhibited very different germination rates and hyphal branching capacity. As can be seen in Table 1, strains P1 and 425 had a lower germination rate and reduced virulence. The presence of branched hyphae did not affect the aggressiveness (Table 1, compare strains 630 and 616). Interestingly, the two strains isolated on tomato (T4 and T8) exhibited similar morphological characteristics, but produced very different symptoms on grapevine. Both were equally virulent when placed on their original host (tomato), and both of these strains penetrated grapevine leaves in a similar fashion. Even though T4 and T8 penetrated a similar number of leaf cells 24 h after inoculation, Evans blue staining for dead cells at 48 h postinoculation revealed that T8 generated larger necrotic lesions than did T4 (Fig. 2A). In fact, by that time, the number of cells killed under the action of T4 never exceeded one to three cells at one site, and these necrotic lesions always extended more slowly than the ones generated by T8. They corresponded to the small black dots described above.

Differential accumulation of secondary metabolites. To explain why the *B. cinerea* isolate T8 was able to spread through grapevine leaf tissues at a faster rate than T4 while both strains had penetrated the epidermis in the same manner, we first looked at the production of defense molecules by the plant in response to these two strains.

The first defense reaction we looked at was the production of phenolics. One of which, a hydroxystilbene phytoalexin named resveratrol (30), exhibits a blue autofluorescence when observed under the UV fluorescent microscope (3). Figure 2B shows that many cells of a leaf inoculated with T4 accumulated blue fluorescent compounds 48 h after inoculation. They appeared very clearly on the red fluorescent background due to the chloroplasts of healthy cells. The cells mainly involved in the production of secondary

Fig. 3. Time course changes in chitinase activity of grapevine leaves inoculated with two strains of *Botrytis cinerea*. In vitro-cultivated grapevine plants were inoculated with either isolate (T4 or T8), treated with unused conidia suspension medium (SM), or not treated (C). At different times after inoculation, the leaves were ground and their chitinase activity estimated (FW = fresh weight). Each dot represents the average value of three separate experiments. Standard deviations do not appear on the graph, since they are smaller than the dot symbols.

Fig. 4. Changes in acidic chitinase isoform pattern in grapevine leaves inoculated with two strains of *Botrytis cinerea*. In vitro-cultivated grapevine plants were inoculated with either isolate (T4 or T8), treated with unused conidia growth medium (SM), or not treated (Control). Chitinase isoforms were extracted from the leaves at the indicated times (expressed in days at the bottom) and separated by native polyacrylamide gel electrophoresis under basic conditions. They were then visualized in an overlay gel containing glycol chitin, and activity bands appeared as dark spots on a white background. Their designations are indicated on the left. Similar isoform patterns were observed in two separate assays.

metabolites formed a ring around the dead cells that appeared black under the UV fluorescent microscope. Leaves infected by the more aggressive strain T8 displayed larger dead areas as already determined by the Evans blue staining of Figure 2A and lacked a ring of cells having accumulated blue fluorescent compounds.

Differential biosynthesis of chitinases and β -1,3-glucanases. Chitinases (8,9,40) and β -1,3-glucanases (8,39) are two other defense products known to be generated by grapevine plants. A time course study of the variations in chitinase activity of grapevine leaves inoculated by T4 or T8 revealed that T4 elicited the biosynthesis of chitinases at a faster rate and to a greater extent than did T8 (Fig. 3). The leaf chitinase activity increased threefold 48 h after inoculation by T4 and reached a maximum 1 day later. In contrast, no significant increase in chitinase activity could be seen in grapevine leaves 48 h after inoculation with T8, but a pronounced increase was detected by day 3. The response was only slightly weaker than the one obtained during the encounter with T4. The difference in timing of the response may, thus, have been a more important factor for the expression of a higher tolerance, as T8-infected leaves were totally rotted by day 4. Replacing the medium normally used to grow the fungus as liquid culture on grapevine leaves did not lead to any significant change in chitinase activity (Fig. 3).

Since about 75% of the chitinase activity expressed by grapevine leaves is due to acidic isoforms (9), the acidic isoforms expressed by leaves inoculated by T4 or T8 were resolved by native polyacrylamide gel electrophoresis under basic conditions and visualized by zymography. As shown in Figure 4, the two chitinase isoforms constitutively expressed (CHV 6 and CHV 11) on uninfected grapevine leaves remained unchanged after infection with the two *B. cinerea* isolates. New chitinase isoforms (CHV 7, CHV 9, and CHV 12) accounted for the rise in chitinase activity observed after T4 inoculation (Fig. 3). T8 elicited the production of the same isoforms as T4, but to a lesser extent (Fig. 4).

β -1,3-Glucanases followed the same induction pattern as chitinases. No glucanase activity was detected in grapevine leaves during the first 24 h postinoculation (Fig. 5). On days 2 and 3, this activity rose more sharply in leaves inoculated with T4 than in the ones inoculated with T8. The medium normally used to grow the fungus as liquid culture did not affect the leaf glucanase activity under the same experimental conditions.

Induction of grapevine defenses by *B. cinerea* culture fluids. The differential induction of grapevine defenses by the two *B.*

Fig. 5. Time course variations of β -1,3-glucanase activity in grapevine leaves inoculated with two strains of *Botrytis cinerea*. In vitro-cultivated grapevine plants were inoculated with either T4 (black bars) or T8 (white bars). At different times after inoculation, the leaves were ground and their β -1,3-glucanase activity estimated (FW = fresh weight). Leaves without treatment or treated with unused growth medium gave undetectable glucanase levels. 1 U = 1 μ g of glucose equivalent released per min. nd = Nondetectable.

cinerea isolates may be due to substances secreted by these two strains. When the culture fluids produced by T4 and T8 were vacuum-infiltrated into grapevine leaves, the results mimicked the induction pattern of the isolates (Fig. 6). The T4 fluid stimulated chitinase activity fourfold, whereas T8 fluid or unused conidial growth medium displayed lower stimulations (2 $\times$) by day 3. The kinetics of chitinase induction by these two culture filtrates were very similar to the induction profiles stimulated by living strains (Fig. 3). None of these culture fluids caused apparent tissue damage.

Several researchers have demonstrated that *B. cinerea* isolates differ in secretion of polygalacturonases, acid proteases, and laccases (43). Table 2 shows that T4 and T8 did not significantly differ in the production of these enzymes when grown on a synthetic medium.

DISCUSSION

The results presented in this study demonstrate that various *B. cinerea* isolates differ in their virulence toward grapevine. A similar conclusion had been drawn on French bean (45), on apple (47), and on grape, strawberry, carrot, potato, and apple (10). Our findings show that two isolates from field-grown grapevine were virulent on vitropplants of this species, while isolates from other plant species were avirulent. A similar finding was found with roses (37), in which isolates obtained from greenhouse-grown roses were more virulent on rose petals than those obtained from gerbera flowers or pea leaves. Still, our *B. cinerea* isolate T8 collected on tomato generated spreading lesions on grapevine, while another tomato isolate, T4, was less aggressive toward this plant.

The hypersensitive response is one of the most efficient mechanisms used by plants to resist pathogen infection. It involves the

Fig. 6. Induction of grapevine chitinase activity by the culture filtrates of two strains of *Botrytis cinerea*. Grapevine leaves were either not treated (C) or vacuum-infiltrated with the culture filtrates of either isolate (T4 and T8) or with unused growth medium (GM). Leaf chitinase activity was measured at different times after infiltration (FW = fresh weight). Each dot represents the average value of three separate experiments. Standard deviations sometimes do not appear on the graph, as they may be smaller than the dot symbols.

TABLE 2. Secretion of polygalacturonase, acid protease, and laccase by two strains of *Botrytis cinerea* grown on a liquid synthetic medium

Isolate	Polygalacturonase activity (U/g dry wt) ^a	Protease activity (U/mg dry wt) ^b	Laccase activity (U/mg dry wt) ^c
T4	54 ± 30	0.93 ± 0.17	1.3 ± 0.4
T8	107 ± 38	0.73 ± 0.02	1.1 ± 0.5

^a 1 U = 1 μ mol of reducing unit released per min.

^b 1 U = 1 μ g of equivalent pepsin.

^c One unit of activity (U) was defined according to Grassin and Dubourdieu (17).

rapid death of a few cells around the intruder and the accumulation of a large array of defense responses to limit further pathogen development (26). The current study suggests that grapevine leaves undergo a hypersensitive-like response when challenged with the less virulent strain of *B. cinerea*, T4. Several defense compounds, secondary metabolites, chitinases, and β -1,3-glucanases, were produced around a very localized group of dead cells. It seems likely, however, that the cells died as a result of pathogen penetration, as the number of cells staining positive with Evans blue was roughly the same as the number of epidermal cells perforated by the fungus. Furthermore, no evidence of hypersensitive cell death or even cytotoxicity could be observed after infiltrating the elicitor-active fungal culture filtrates. These results are in agreement with a study showing that a culture filtrate of a *B. cinerea* strain virulent on grapevine did not elicit any defense response from grapevine cell cultures, while a cellulase fraction of *Trichoderma viride*, a *B. cinerea* biological control agent, elicited a hypersensitive-like response on grapevine cells (3). A similar hypersensitive-like reaction has also been suggested to occur on primary French bean leaves (45), in which the resistance of bean leaves to an isolate of *B. cinerea* correlated with phytoalexin accumulation. A virulent isolate did not elicit this response. van den Heuvel and Grootveld (45) also showed that phytoalexins mainly accumulated around dead cells, a conclusion also reached by a study showing accumulation of resveratrol around the lesions made by an avirulent *B. cinerea* isolate on grapevine leaves (28) and is in agreement with our data. Phytoalexins have, however, been suggested to be unable to account alone for resistance phenotype toward *Botrytis* (45). *B. cinerea* has been shown to secrete polyphenol oxidases capable of detoxifying the grapevine phytoalexin resveratrol (2). We find that other defense factors such as chitinases and β -1,3-glucanases are generated along with phytoalexins. One chitinase isoform expressed by ripe grape berries (CHV 5) has recently been purified and shown to exhibit strong anti-*Botrytis* properties by inhibiting conidia germination and germ tube elongation (8). This particular isoform is, however, not expressed by grapevine leaves that express the three other berry isoforms, CHV 1, CHV 11, and CHV 12 (8,9). The effect of other leaf chitinases on *B. cinerea*, either alone or in combination, is currently under investigation.

Salicylic acid is a substance involved in many plants in regulating expression of their defense machinery (7). In grapevine, salicylic acid controls the production of several chitinase isoforms in leaves (9) and berries (8). Interestingly, these authors showed that salicylic acid stimulates the activity of the same set of chitinase isoforms in grapevine leaves as does the less virulent *B. cinerea* strain T4 in this study (i.e., CHV 7, CHV 9, and CHV 12). Salicylic acid may, thus, participate in the resistance reaction of grapevine leaves to *Botrytis*. This hypothesis is supported by the observation that selected strains of nonpathogenic rhizobacteria induce a systemic resistance in bean to *B. cinerea* via their ability to secrete salicylic acid (6).

Under natural conditions, *B. cinerea* develops latent infections on grapes that will expand rapidly when environmental conditions are favorable (34). A model for the expansion of lesions was established based on the diffusion through the host plant tissue of secretions from *B. cinerea* (22). According to this model, these secretions are phytotoxic at high concentrations around the hyphal tips, but elicit defense responses in surrounding healthy tissues (24). A glucan and heteropolysaccharide fractions secreted by *B. cinerea* elicited phytoalexin production in bean (25), and a similar glucan fraction stimulated stilbene synthase activity in grapevine cell suspensions (30). In another study, phytotoxic effects were attributed to *B. cinerea* pectin-degrading enzymes, which have been held responsible for middle lamella degradation and plasmalemma rupture (23). Finally, a crude mycelial extract of *B. cinerea* was shown to induce chitinase and β -1,3-glucanase production in bean cells and protoplast cultures (27). None of the above studies, however, established whether the eliciting, or phytotoxic,

fractions under study originated from aggressive or nonaggressive strains of *Botrytis* and they actually corresponded to the same substances. This could be of some relevance, since our results suggest that less virulent strains secrete greater amounts of an eliciting fraction. This fraction was not phytotoxic at the concentration that was tested. Additional studies will now be required to determine the exact chemical nature of the active elicitor components secreted by T4, and thus contribute to a better understanding of the interaction between *Botrytis* and grapevine.

ACKNOWLEDGMENTS

This work was supported by a grant from Europôl'Agro. We thank S. Brunet, N. Cheutin, C. Hachet, C. Hamelin, C. Pierrard, and F. Wrissez for their technical assistance. We also thank Y. Brygoo for providing the *Botrytis cinerea* isolates used in this study.

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