Antifungal Activity of Chitosan on Two Postharvest Pathogens of Strawberry Fruits

Ahmed El Ghaouth, Joseph Arul, Jean Grenier, and Alain Asselin

First and second authors: Département de science et technologie des aliments et Centre de recherche en horticulture, Université Laval, Québec, G1K 7P4, Canada; third and fourth authors: Département de phytologie, Université Laval, Québec, G1K 7P4, Canada.

This research was supported by the Conseil des recherches en pêches et agro-alimentaire (CORPAQ). We thank Jean Trudel for his collaboration and Louise Laroche for typing the manuscript.

Address correspondence to J. Arul.

Accepted for publication 5 November 1991.

ABSTRACT

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

Effect of chitosan coating on decay of strawberry fruits held at 13 C was investigated. Strawberry fruits were inoculated with spore suspensions of *Botrytis cinerea* or *Rhizopus stolonifer* and subsequently coated with chitosan solutions (10 or 15 mg/ml). After 14 days of storage, decay caused by *B. cinerea* or *R. stolonifer* was markedly reduced by chitosan coating. Decay was not reduced further when the concentration of chitosan coating was increased from 10 to 15 mg/ml. Coating intact strawberries with chitosan did not stimulate chitinase, chitosanase, or β-1,3-glucanase activities in the tissue as revealed by polyacrylamide gel assays. Chitosan, when applied on freshly cut strawberries, however, stimulated acidic chitinase activity. Chitosan was very effective in inhibiting spore germination, germ tube elongation, and radial growth of *B. cinerea* and *R. stolonifer* in culture. Furthermore, chitosan at a concentration greater than 1.5 mg/ml induced morphological changes in *R. stolonifer*. Mechanisms by which chitosan coating reduced the decay of strawberries appear to be related to its fungistatic property rather than to its ability to induce defense enzymes such as chitinase, chitosanase, and β-1,3-glucanase.

Additional keywords: *Fragaria* sp., glucanohydrolase, gray mold.

Postharvest decay represents major losses in the horticultural industry. During storage and shipment of strawberries (*Fragaria* × *ananassa* Duchesne), decay losses are mainly caused by *Botrytis cinerea* Pers.: Fr. (gray mold rot) and *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. (soft rot) (4). These losses could be reduced to a certain extent by minimizimg mechanical damages, maintaining the natural resistance of the produce, and storing at optimal conditions such as low temperature and high CO_2 (15–20%) atmosphere (8,18,19,24). Although a high CO_2 concentration is effective in controlling decay and delaying ripening, it may cause discoloration of the tissue and off flavor of the fruit when it exceeds the tolerance limit (8,18).

Application of fungicides is by far the most effective method to control postharvest diseases (12,25). However, chemical control programs face imminent problems: first, there are reports of an increasing number of fungicide-resistant strains of postharvest pathogens (26); and second, a number of commonly used fungicides such as benomyl are under review in many countries due to health risk concerns. Thus, there is a growing need to develop alternative approaches for control of postharvest diseases; one approach that is being actively pursued involves the use of bioactive substances (28).

Chitosan, a high molecular weight cationic polysaccharide, has been shown to be fungicidal against several fungi (1,6,11,14,27). Allan and Hadwiger (1) reported that chitosan (1,000 μg/ml) was effective in reducing the radial growth of most fungi tested, except those containing chitosan as a major cell wall component (i.e., Zygomycetes). Inhibitory effect of chitosan also was demonstrated with soilborne phytopathogenic fungi (27). Stössel and Leuba (27) showed that the inhibitory activity of chitosan was higher at pH 6.0 (pK_a value of chitosan = 6.2) than at pH 7.5, when most amino groups are in the free base form. Kendra and Hadwiger (15) demonstrated that the maximal antifungal and pisatin-inducing activities of chitosan were exhibited by chitosan oligomers of seven or more residues.

Chitosan also is known to be a potential elicitor of many plant defense responses, including the accumulation of chitinases (20), synthesis of proteinase inhibitors in tomato leaves (30), lignification in wheat leaves (23), and induction of callose synthesis (13). Chitosan appears to play a dual function, by interferring directly with fungal growth and also by activating several biological processes in plant tissues. In addition, due to its polymeric nature, chitosan can form films permeable to gases (2). Hence, chitosan has the potential as an edible antifungal coating material for postharvest produce. Recent investigations on chitosan coating of tomatoes have shown that it delayed ripening by modifying the internal atmosphere and that it reduced decay (7).

The objectives of this research were to assess the effect of chitosan coating on decay of strawberry fruits, to determine if chitosan coating directly affects fungal growth, and to determine if chitosan induces defense enzymes such as chitinase, chitosanase, or β-1,3-glucanase activities in strawberry tissue.

MATERIALS AND METHODS

Materials. Crab-shell chitosan was purchased from ICN Biochemical Inc. (Cleveland, OH) and ground to a fine powder. The purified chitosan was prepared by dissolving chitosan in 0.25 N HCl, and the undissolved particles were removed by centrifugation (15 min, 10,000 *g* at 24 C). The viscous solution was then neutralized with 2.5 N NaOH (pH 9.8). Precipitated chitosan was collected by centrifugation, washed extensively with deionized water to remove the salts, and subsequently lyophilized.

B. cinerea and *R. stolonifer* were isolated from strawberries and maintained on potato-dextrose agar (PDA). Strawberries (cv. Chandler grown locally) were harvested and rapidly cooled to below 7 C using the vapors of dry ice. Berries were sorted on the basis of size, color (75% full red color), and absence of physical damage, and were randomly divided into lots of 70 fruits.

Chemicals used for electrophoresis and protein molecular mass markers were purchased from Bio-Rad (Mississauga, Ontario, Canada). Calcofluor white M2R (CI 40622) and Triton X-100

were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade.

Decay. Chitosan solutions (10 and 15 mg/ml) were prepared by dissolving chitosan in 0.25 N HCl and adjusting the pH to 5.6 with 2 N NaOH. Strawberry fruits were inoculated by dipping in a solution of 0.1% (v/v) Tween 80 containing 2×10^5 conidia per milliliter of *B. cinerea* or *R. stolonifer* and were allowed to air dry at 20 C for 2 h. Inoculated berries were then individually dipped either in the chitosan solution (10 or 15 mg/ml) with 0.1% (v/v) Tween 80 or in sterile deionized water (pH 5.6) containing 0.1% (v/v) Tween 80. Treatments consisted of four replicates of 70 berries each and were arranged in a randomized complete block design. After air drying at 20 C for 2 h, berries were stored at 13 C in plastic containers with a continuous flush of humidified air (95% relative humidity [RH]). Strawberries were evaluated daily for symptoms, and spoiled fruits were immediately discarded to avoid secondary infection. The experiment was repeated four times. Pooled data were analyzed by analysis of variance procedures.

Spore germination. Cultures of 10-day-old *B. cinerea* and 3-day-old *R. stolonifer* were flooded with sterile distilled water containing 0.1% (v/v) Tween 80 and were gently agitated to remove the spores. Spore suspensions were adjusted with sterile distilled water to 2×10^5 conidia per milliliter. PDA plates amended with chitosan were prepared as follows: purified chitosan was dissolved in 0.25 N HCl, and the pH was adjusted to 5.6 with 2 N NaOH (27). The solution was autoclaved and subsequently added to sterile molten PDA to obtain chitosan concentrations of 0, 0.75, 1.5, 3.0, or 6.0 mg/ml. Aliquots of 20 ml of this solution were immediately dispensed into 9-cm-diameter polystyrene petri plates. Two 50-μl aliquots of the spore suspensions were pipetted onto each PDA plate amended with chitosan. Four replicates of six plates were used for each fungus at each concentration of chitosan. Control plates contained PDA with the pH adjusted to 5.6. Inoculated plates were incubated at 15 C for 24 h. Germination of 100 spores per plate was determined microscopically. A spore was considered germinated when the length of the germ tube equaled or exceeded the length of the spore. At each concentration, the germ tube length of 70–90 spores was measured with an ocular micrometer. The experiment was a completely randomized design, and the test was repeated twice. For variance and linear regression analyses, a logarithmic transformation was applied for chitosan concentration and percentage of inhibition of germination and germ tube length relative to the control. Because the results were similar for both tests, only the results from the first test are presented.

Radial growth. PDA plates containing 0, 0.75, 1.5, 3.0, or 6.0 mg/ml of purified chitosan were prepared as described above and were seeded with 6-mm-diameter agar plugs taken from the margin of 4-day-old *B. cinerea* or 2-day-old *R. stolonifer* cultures. Four replicates of eight plates were used for each fungus at each concentration of chitosan, and the plates were incubated in the dark at 24 C. Growth measurements were determined when the growth on the control (0 mg/ml of chitosan) reached the edge of the plate. The experimental design was a randomized complete block. The test was repeated twice, and each test was analyzed separately. Data were analyzed by analysis of variance; then, linear regression analysis with logarithms of chitosan concentration and percentage of inhibition of growth was performed. Because the results were similar for both tests, only the results from the first test are presented.

Crude enzyme preparation. Strawberry fruits were surface-sterilized by dipping in a solution of 1% sodium hypochlorite for 1 min. Whole strawberries were coated with 10 or 15 mg/ml of chitosan as described above. Other berries were carefully split into halves. On the cut surface, 50 μl of chitosan (1 mg/ml) or sterile water (pH 5.6) was applied. Whole and cut berries treated with chitosan and their controls were stored at 13 C in plastic containers with a continuous flush of humidified air (95% RH) for 12 or 48 h. For each treatment, triplicates of 10 fruits were used. A weighed portion from each treatment of strawberries (5 g) was homogenized at 4 C in 10 ml of 50 mM sodium phosphate buffer (pH 5.0) in a Sorval Omni-mixer at maximum speed. The homogenate was centrifuged at 4 C (15 min, 15,000 *g*), and the supernatant was used as the crude enzyme preparation. Protein concentration in filtered supernatant was determined with the Bio-Rad protein assay kit (9). The test was repeated three times.

Polyacrylamide gel electrophoresis (PAGE) and chitinolytic activity. The sodium dodecyl sulfate (SDS)-PAGE was performed at pH 8.9 according to Laemmli (16) by using a 15% (w/v) polyacrylamide gel containing 0.01% (w/v) glycol chitin as substrate and 0.1% (w/v) SDS. The stacking gel contained 5% (w/v) polyacrylamide and 0.1% (w/v) SDS. The crude enzyme samples were boiled for 2 min in 10% (w/v) sucrose and 2.0% (w/v) SDS in 125 mM Tris-HCl (pH 6.7). Electrophoresis was run at room temperature for 1.5 h at 20 mA. After electrophoresis, the gel was incubated overnight at 37 C on a rotating apparatus in 200 ml of 100 mM sodium acetate buffer (pH 5.0) containing 1.0% (v/v) purified Triton X-100. At the end of the incubation period, the gel was stained with 0.01% (w/v) Calcofluor white M2R in 0.3 M Tris-HCl (pH 8.9) for 5 min, and destained by incubating the gel in 100 ml of distilled water with gentle shaking for 2 h (29). Lytic zones were visualized by placing the gel on a Chromato-Vue C-62 transilluminator (UV Products, San Gabriel, CA) and photographed with Polaroid film type 55 P/N by using UV-haze and a 02-orange filter (29). Chitinase activity also was analyzed after two-dimensional electrophoresis gels with native PAGE at pH 4.3 or 8.9 in the first dimension and SDS-PAGE in the second dimension (29).

Detection of chitosanase and β-1,3-glucanase activities. Chitosanase activity after SDS-PAGE was detected by Calcofluor white M2R staining after lysis of glycol chitosan as substrate in the gel matrix (9). Renaturation of enzyme activity after SDS-PAGE was in 100 mM sodium acetate (pH 5.0), containing 1.0% (v/v) purified Triton X-100 (9), for 18 h at 37 C. β-1,3-Glucanase activity after native PAGE for acidic (Davis system) and basic (Reisfeld system) proteins was performed according to Côté et al (5). β-1,3-Glucanase activity was detected after hydrolysis of laminarin (1.5 mg/ml) as substrate in the gel matrix. Detection was with aniline blue staining as described for tobacco pathogenesis-related proteins (5).

Antifungal activity. Antifungal activity of strawberry extracts was evaluated with *B. cinerea* and *R. stolonifer*. PDA plates were inoculated with spore suspensions (2×10^5 spores per milliliter) of 2-day-old *R. stolonifer* or 10-day-old *B. cinerea* cultures. To allow for spore germination and initial hyphal extension, inoculated plates were incubated at 27 C for 1 and 3 days for *R. stolonifer* and *B. cinerea*, respectively. Extracts (20 μl) of intact fruits treated with 0, 10, or 15 mg/ml of chitosan and cut fruits treated with 0 or 1 mg/ml of chitosan as well as their controls (boiled for 10 min) were applied as drops on agar in advance of the growing fungi. Plates were incubated at 27 C. Inhibition of fungal growth was monitored in intervals of 24 h after the onset of the treatment.

TABLE 1. Effect of chitosan coating on incidence of gray mold and soft rot of strawberries

Treatment[a]	Storage (days)	Percentage of decay[b]	
		Botrytis cinerea	*Rhizopus stolonifer*
Control	7	34.0 (±0.5)	39.1 (±0.3)
	14	72.7 (±0.6)	78.0 (±0.6)
Chitosan (10 mg/ml)	7	9.2 (±0.2)	10.0 (±0.2)
	14	34.6 (±0.4)	37.0 (±0.6)
Chitosan (15 mg/ml)	7	7.0 (±0.5)	10.2 (±0.4)
	14	30.0 (±0.7)	32.1 (±0.7)

[a] Inoculated berries were individually dipped either in chitosan solution (10 or 15 mg/ml) or in sterile deionized water containing 0.1% (v/v) Tween 80, air-dried, and then stored at 13 C.

[b] Percentage of infected strawberries was based on four replicates of 70 fruits each. Values in the parentheses are standard errors of the mean.

RESULTS

Decay of strawberries. Chitosan coating was effective in reducing decay of strawberry fruits caused by *B. cinerea* or *R. stolonifer*. Signs of infection in chitosan-coated fruits appeared after 5 days of storage at 13 C, whereas in the control treatment, infections were visible after 1 day. After 14 days of storage, chitosan coating at 15 mg/ml reduced decay of strawberries caused by both fungi by more than 60% (Table 1). There was no significant decrease in decay when the concentration of chitosan was increased from 10 to 15 mg/ml. Coated fruits ripened normally and did not show any apparent sign of phytotoxicity.

Effect of chitosan on spore germination and radial growth. Chitosan at pH 5.6 markedly reduced spore germination and germ tube elongation of *B. cinerea* and *R. stolonifer* (Table 2). At the highest concentration (6 mg/ml), chitosan inhibited spore germination and germ tube elongation of *B. cinerea* and *R. stolonifer* by more than 90 and 75%, respectively. Chitosan appeared to be more effective in inhibiting spore germination and germ tube elongation of *B. cinerea* than *R. stolonifer*. Although *R. stolonifer* was less sensitive than *B. cinerea* to chitosan, concentrations higher than 1.5 mg/ml induced morphological changes characterized by excessive hyphal branching (Fig. 1A) as compared to the control (Fig. 1B).

Chitosan inhibited the radial growth of both pathogens, with a marked effect at higher concentrations, more so with *B. cinerea* than *R. stolonifer* (Table 3). At concentrations higher than 1.5 mg/ml, chitosan induced abnormal aerial hyphal growth of *B. cinerea* and *R. stolonifer* on the agar surface and also reduced the overall production of sporangia by *R. stolonifer* (data not shown).

Effect of chitosan on chitinase, chitosanase, β-1,3-glucanase activity and antifungal activity. Crude extracts of chitosan-coated strawberries stored for 12 and 48 h and analyzed for chitinase activity after SDS-PAGE exhibited two bands at 80 and 30 kDa with chitinolytic activity (data not shown). Those same bands also were present with similar intensity in the control extracts (data not shown). Extracts from freshly cut strawberries treated with chitosan showed three major bands estimated at 80, 48, and 30 kDa and one minor band with chitinase activity at 35 kDa (Fig. 2B,D), whereas extracts of water-treated controls exhibited only one major band with chitinolytic activity (Fig. 2A,C). The intensity of lytic bands found in strawberries stored for 12 h was similar to those of strawberries stored for 48 h (Fig. 2A,B vs. D,C). Similar induction of chitinase also was observed with cut strawberries of different maturity (data not shown). Extracts were analyzed in two-dimensional gels with the first dimension of native PAGE for acidic (Fig. 3, arrow a) or basic proteins (arrow b) and SDS-PAGE in the second dimension. Three acidic chitinases (Fig. 3, arrowheads 1–3) were observed with extracts of the cut strawberries treated with chitosan. The acidic bands were estimated at 80, 48, and 30 kDa. The chitinase band at 35 kDa, detected after one-dimensional SDS-PAGE (Fig. 3B), was not detected after two-dimensional separation. The upper acidic band (Fig. 3, arrowhead 1) also was observed in extracts of the water-treated control (data not shown). In the Reisfeld system designed to separate basic proteins, no chitinase activity could be detected in either extract.

Extracts of strawberries also were analyzed for chitosanase after SDS-PAGE and for β-1,3-glucanase activity under native conditions for acidic and basic proteins. No chitosanase or β-1,3-glucanase activity, however, was detected by these gel assays. None of the extracts from cut or whole strawberries treated with

TABLE 2. Effect of chitosan on spore germination and germ tube length of *Botrytis cinerea* and *Rhizopus stolonifer*

Chitosan (mg/ml)	Percentage of inhibition			
	B. cinerea		*R. stolonifer*	
	Germination[a,b]	Germ tube length[c,d]	Germination[a,b]	Germ tube length[c,d]
0	0	0	0	0
0.75	35.2	30.7	7.2	2.4
1.5	57.2	55.2	18.2	13.6
3.0	93.0	85.3	68.5	60.3
6.0	98.7	93.7	75.5	76.8

[a] Germination of 100 spores per plate on four replicates of six plates each were determined after 24 h at 15 C.

[b] Regression equations of log percentage of inhibition of germination (Y) on log chitosan concentration (X) are as follows: *B. cinerea* $Y = 0.52X + 0.1$, $r = 0.96$; *R. stolonifer* $Y = 1.21X - 2.56$, $r = 0.96$. Regression coefficients are significant at $P \leq 0.05$.

[c] Each value represents the average of 70–90 spores.

[d] Regression equations of log percentage of inhibition of germ tube length (Y) on log chitosan concentration (X) are: *B. cinerea* $Y = 0.55X - 0.035$, $r = 0.96$; *R. stolonifer* $Y = 1.71X - 4.41$, $r = 0.96$. Regression coefficients were significant at $P \leq 0.05$.

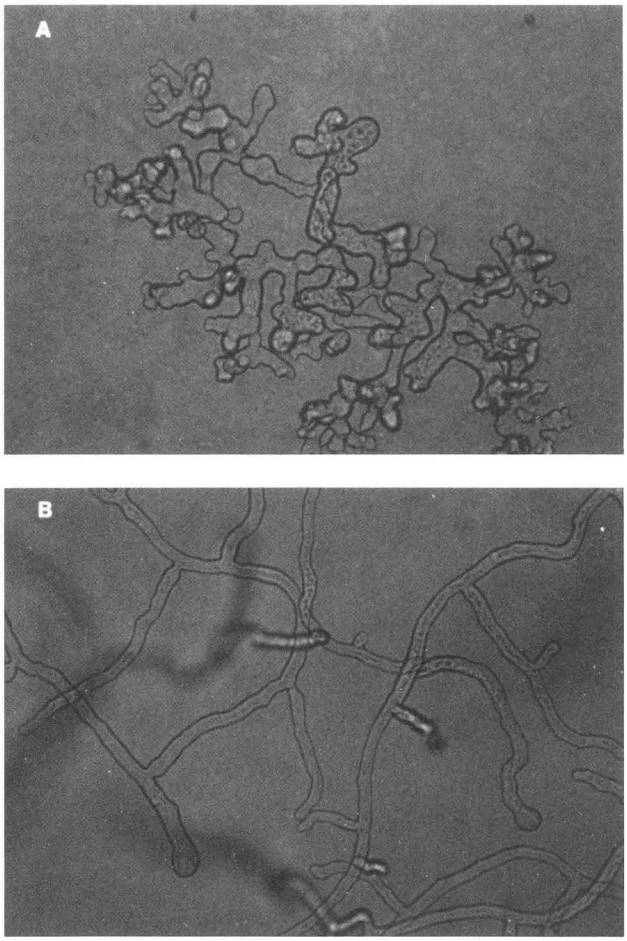

Fig. 1. Effect of chitosan on hyphal morphology of *Rhizopus stolonifer* grown on potato-dextrose agar amended with **A,** chitosan or **B,** nonamended.

TABLE 3. Effect of chitosan on the radial growth of *Botrytis cinerea* and *Rhizopus stolonifer* on potato-dextrose agar (PDA)

Chitosan (mg/ml)	Percentage of inhibition of radial growth[a]	
	B. cinerea	*R. stolonifer*
0.75	38.1	4.7
1.5	59.3	20.8
3.0	77.4	58.4
6.0	95.5	71.5

[a] Measurement of radial growth was performed 7 and 3 days after PDA plates were inoculated with *B. cinerea* or *R. stolonifer*, respectively. Regression equations of log of inhibition of radial growth (Y) on log of chitosan concentration (X) are as follows: *B. cinerea* $Y = 0.44X + 0.36$, $r = 0.98$; *R. stolonifer* $Y = 1.32X - 2.99$, $r = 0.95$. Correlation coefficients are significant at $P \leq 0.05$.

chitosan inhibited the growth of *B. cinerea* and *R. stolonifer* (data not shown).

DISCUSSION

Chitosan coating was effective in reducing decay of strawberry fruits caused by *B. cinerea* or *R. stolonifer*. When tested in vitro, chitosan inhibited spore germination and radial growth of both fungi. Complete inhibition, however, was not achieved even at a concentration of 6 mg/ml, indicating that chitosan is fungistatic rather than fungicidal. The observed inhibition of decay could, in principle, be due to either the fungistatic property of chitosan and/or to its ability to induce defense enzymes.

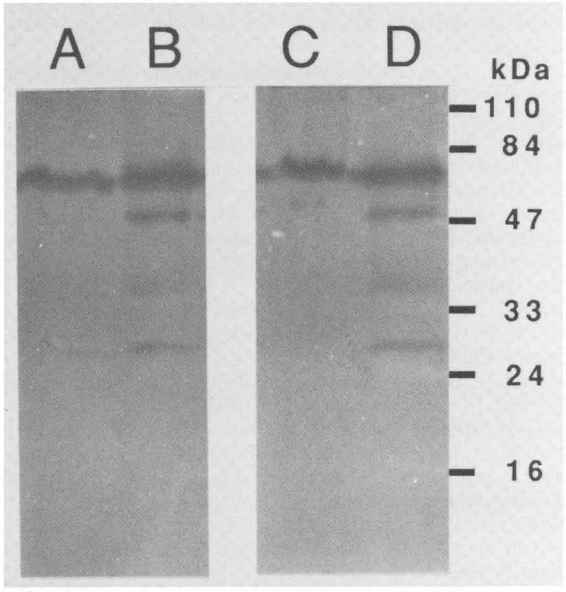

Fig. 2. Chitinase activities detected in strawberry fruits after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Extracts of cut strawberries treated with deionized water and stored for **A,** 12 h and **C,** 48 h or treated with chitosan and stored for **B,** 12 and **D,** 48 h were subjected to SDS-PAGE in polyacrylamide slab gel containing 0.01% (w/v) glycol chitin as substrate for chitinase activity. Chitinase activity was visualized by staining with Calcofluor white M2R.

Plant glucanohydrolases such as chitinase, chitosanase, and β-1,3-glucanase may be implicated in plant defense against pathogenic fungi (3). Mauch et al (21) have demonstrated that chitinase and β-1,3-glucanase were effective in inhibiting the in vitro growth of several fungi. The role of glucanohydrolases in disease resistance, however, has not been definitively demonstrated (3). In the present study, the inhibition of decay may not be a result of the stimulation of defense enzymes by chitosan in intact fruits. Indeed, coating of intact fruits with chitosan did not result in a stimulation of chitinase, chitosanase, or β-1,3-glucanase. In addition, extracts from coated fruits did not exhibit any inhibitory activity towards *B. cinerea* and *R. stolonifer*.

Stimulation of chitinase activities was observed, however, when chitosan was applied directly on freshly cut fruits. This suggests that close contact with tissue is presumably required for the elicitation. Induction of chitinase and β-1,3-glucanase activities (14,20,22) and the elicitation of phytoalexins (14,15) by chitosan also have been reported in excised pea pods. The inability of chitosan coating to stimulate chitinase in intact fruits could quite possibly be due to the limited intimate interaction between the coating material and the tissue. Strawberry cuticle, which is nonporous, may physically separate chitosan from the tissue and consequently prevent chitosan from inducing chitinases. This possibility should not be disregarded, especially because membranes with large pores (0.4 μm), which can allow macromolecule interchanges when used to prevent direct contact between the elicitor and the exposed pea tissue, have been shown to prevent an elicitor from inducing defense responses (22).

Chitinases of high molecular mass (>40 kDa) are usually reported in microorganisms (bacteria and fungi) but not in higher plants (3). The two chitinases (estimated at 48 and 80 kDa) found after SDS-PAGE are of plant origin, because the same bands could not be detected in the mycelium and culture filtrates of *B. cinerea* and *R. stolonifer*. In addition, similar bands were found in strawberry leaf extracts (data not shown). The lack of response of test fungi to chitinases from cut berries treated with chitosan extracts may be due, at least in part, to their cell wall structure. *R. stolonifer*, for instance, contains chitosan in its cell wall, which can interact with chitin and render it less accessible to hydrolases. In *B. cinerea*, chitin may be protected by β-1,3-glucans, thus β-1,3-glucanase may be required to render chitin accessible to chitinase (21).

Based on the results from the in vitro studies, inhibition of decay is possibly due to the fungistatic property of chitosan. We

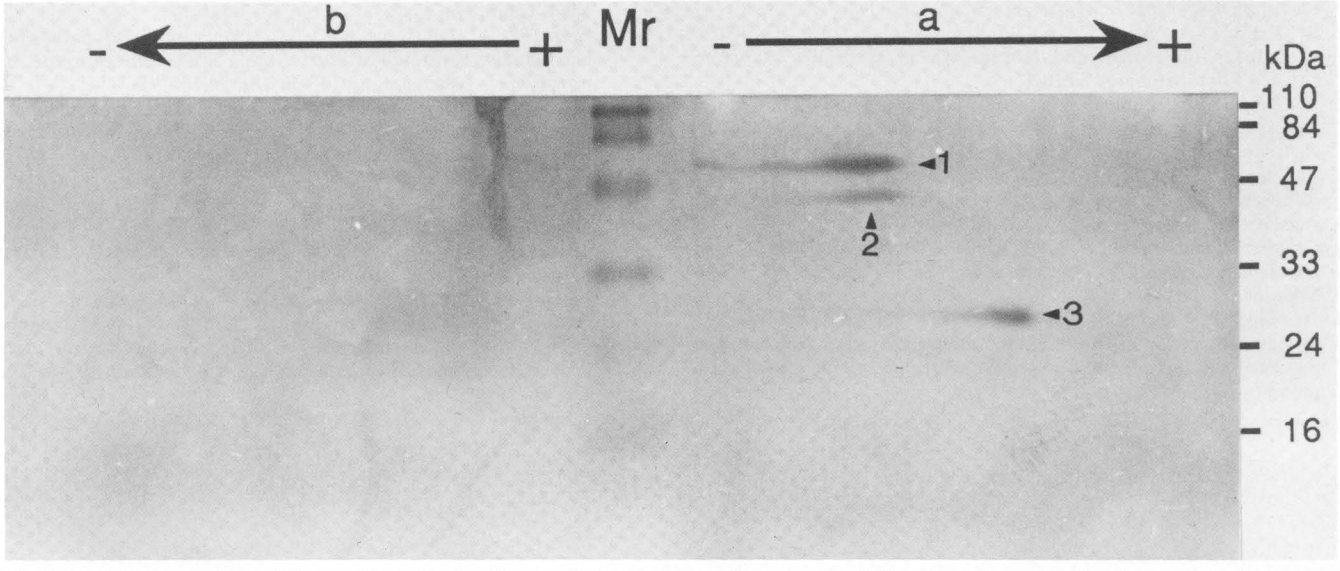

Fig. 3. Chitinase activity detected in strawberry fruits after separation in a two-dimensional gel. Extracts of cut strawberries treated with chitosan were first subjected to native gel electrophoresis in polyacrylamide gels (PAGE) for acidic (arrow a) or basic proteins (arrow b). After native PAGE, gel slices were boiled for 5 min in sodium dodecyl sulfate (SDS) buffer and subjected to SDS-PAGE in a 15% (w/v) polyacrylamide SDS gel containing 0.01% (w/v) glycol chitin. Enzyme activity was revealed by Calcofluor white M2R staining. Prestained molecular mass markers (lane Mr) (5 μg in 10 μl) were run in the second dimension. Numbers on the right refer to molecular mass markers (kDa). Arrowheads indicate the position of chitinases.

have demonstrated that chitosan was effective in inhibiting the growth in vitro of two major pathogens of strawberry fruits. The comparison of our results with the data reported in the literature is difficult because of the diversity of fungi used and the different methods used to incorporate chitosan into the growth medium. Although chitosan is known to affect the growth of most fungi, except those containing chitosan as a major cell wall constituent, the mechanism explaining its antifungal action has not been fully elucidated. Recently, two models have been proposed to explain the antifungal activity of chitosan. According to Leuba and Stössel (17), the activity of chitosan is related to its ability to interfere with the plasma membrane function. In the model of Hadwiger and Loschke (10), the interaction of chitosan with fungal DNA and mRNA is the basis of its antifungal effect. Our results do not provide any clue as to the nature of growth inhibition in fungi by chitosan. However, it demonstrates that in the less sensitive fungi such as *R. stolonifer*, chitosan can induce gross morphological alterations. To our knowledge, this is the first report of the effect of chitosan on fungal morphology. These morphological changes are possibly related to the effect exerted by chitosan indirectly on the formation of the hyphal wall. While this remains to be verified, such changes were not observed with *B. cinerea*, indicating that the effect of chitosan could vary with fungi. In conclusion, this study demonstrates the potential of chitosan as an antifungal preservative for strawberry fruits that are quite susceptible to decay caused by *B. cinerea* and *R. stolonifer*. In addition, chitosan potentially is an attractive preservative agent for cut fruits because of the interplay of its antifungal property as well as its ability to stimulate some defense enzymes in the exposed tissue.

LITERATURE CITED

1. Allan, C. R., and Hadwiger, L. A. 1979. The fungicidal effect of chitosan on fungi of varying cell wall composition. Exp. Mycol. 3:285-287.
2. Bai, R. K., Huang, M. Y., and Jiang, Y. Y. 1988. Selective permeabilities of chitosan-acetic complex membrane and chitosan-polymer complex for oxygen and carbon dioxide. Polym. Bull. 20:83-88.
3. Boller, T. 1988. Ethylene and the regulation of antifungal hydrolases in plants. Oxf. Surv. Plant Mol. Cell Biol. 5:145-174.
4. Ceponis, M. J., Cappellini, R. A., and Lightner, G. W. 1987. Disorders in sweet cherry and strawberry shipments to the New York market, 1972–1984. Plant Dis. 71:472-475.
5. Côté, F., Letarte, J., Grenier, J., Trudel, J., and Asselin, A. 1989. Detection of β-1,3-glucanase activity after native polyacrylamide gel electrophoresis: Application to tobacco pathogenesis-related proteins. Electrophoresis 10:527-529.
6. El Ghaouth, A., Arul, J., and Ponnampalam, R. 1990. The effect of chitosan on growth and morphology of *Rhizopus stolonifer*. (Abstr.) Phytopathology 80:1020.
7. El Ghaouth, A., Ponnampalam, R., and Arul, J. 1990. The use of chitosan coating to extend the storage-life of tomato fruits. (Abstr.) HortScience 25:1086.
8. El-Kazzaz, M. K., Sommer, N. F., and Fortlage, R. J. 1983. Effect of different atmospheres on postharvest decay and quality of fresh strawberries. Phytopathology 73:282-285.
9. Grenier, J., and Asselin, A. 1990. Some pathogenesis-related proteins are chitosanases with lytic activity against fungal spores. Mol. Plant-Microbe Interact. 3:401-407.
10. Hadwiger, L. A., and Loschke, D. C. 1981. Molecular communication in host-parasite interactions: Hexosamine polymers (chitosan) as regulator compounds in race-specific and other interactions. Phytopathology 71:756-762.
11. Hirano, S., and Nagao, N. 1989. Effect of chitosan, pectic acid, lysozyme and chitinase on the growth of several phytopathogens. Agric. Biol. Chem. 53:3065-3066.
12. Jordan, V. W. L. 1973. The effects of prophylactic spray programs on the control of pre- and postharvest diseases of strawberry. J. Plant Pathol. 22:67-70.
13. Kauss, H., Jeblick, W., and Domard, A. 1989. The degree 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.
14. Kendra, F. D., Christian, D., and Hadwiger, L. A. 1989. Chitosan oligomers from *Fusarium solani*/pea interactions, chitinase/β-glucanase digestion of sporelings and from fungal wall chitin actively inhibit fungal growth and enhance disease resistance. Physiol. Mol. Plant. Pathol. 35:215-230.
15. Kendra, F. D., and Hadwiger, L. A. 1984. Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum*. Exp. Mycol. 8:276-281.
16. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680-685.
17. Leuba, J. L., and Stössel, P. 1986. Chitosan and other polyamines: Antifungal activity and interaction with biological membranes. Pages 215-222 in: Chitin in Nature and Technology. R. Muzzarelli, C. Jeauniaux, and G. W. Gooday, eds. Plenum Press, New York.
18. Li, C., and Kader, A. A. 1989. Residual effect of controlled atmospheres on postharvest physiology and quality of strawberries. J. Am. Soc. Hortic. Sci. 114:629-634.
19. Maas, J. L. 1981. Postharvest diseases of strawberry. Pages 329-353 in: The Strawberry-Cultivars to Marketing. N. F. Childers, ed. Horticultural Publications, Gainesville, FL.
20. Mauch, F., Hadwiger, L. A., and Boller, T. 1984. Ethylene: Symptom, not signal for the induction of chitinase and β-1,3-glucanase in pea pods by pathogens and elicitors. Plant Physiol. 76:607-611.
21. Mauch, F., Hadwiger, L. A., and Boller, T. 1988. Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combination of chitinase and β-glucanase. Plant Physiol. 88:936-942.
22. Nichols, E. J., Beckman, J. M., and Hadwiger, L. A. 1980. Glycosidic enzyme activity in pea tissue and pea-*Fusarium solani* interactions. Plant Physiol. 66:199-204.
23. Pearce, R. B., and Ride, J. P. 1982. Chitin and related compounds as elicitors of the lignification response in wounded wheat leaves. Physiol. Plant Pathol. 20:119-123.
24. Shewleft, R. L. 1986. Postharvest treatment for extending the shelf life of fruits and vegetables. Food Technol. 40:70-80.
25. Sommer, N. F. 1985. Strategies for control of postharvest diseases of selected commodities. Pages 83-99 in: Postharvest Technology of Horticultural Crops. A. A. Kader, R. F. Kasmire, F. G. Mitchell, M. S. Reid, N. F. Sommer, and J. F. Thompson, eds. Coop. Ext., Univ. Calif., Davis.
26. Spotts, R. A., and Cervantes, L. A. 1986. Populations, pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* in packinghouses. Plant Dis. 70:106-108.
27. Stössel, P., and Leuba, J. L. 1984. Effect of chitosan, chitin and some aminosugars on growth of various soilborne phytopathogenic fungi. Phytopathol. Z. 111:82-90.
28. Takeda, F., Janisiewicz, W. J., Roitman, J., Mahoney, N., and Abeles, F. B. 1990. Pyrrolnitrin delays postharvest fruit rot in strawberries. HortScience 25:320-322.
29. Trudel, J., and Asselin, A. 1989. Detection of chitinase activity after polyacrylamide gel electrophoresis. Anal. Biochem. 178:362-366.
30. Walker-Simmons, M., Hadwiger, L. A., and Ryan, C. A. 1983. Chitosan and pectic polysaccharides both induce the accumulation of the antifungal phytoalexin pisatin in pea pods and antinutrient proteinase inhibitors in tomato leaves. Biochem. Biophys. Res. Comm. 110:194-199.