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Apple (*Malus domestica*) and Tomato (*Lycopersicum esculentum*) Fruits Cell-Wall Hemicelluloses and Xyloglucan Degradation during *Penicillium expansum* Infection

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We characterized the changes in cell-wall hemicellulosic polysaccharides and the hemicellulose-degrading enzymes associated with apple and tomato fruits infected by *Penicillium expansum*. Our results showed a reduction in the molecular mass of hemicelluloses, with this reduction being particularly notable in the xyloglucan associated with *P. expansum* infection. The activation of fungal β-glucanases was also highlighted. Fruit xyloglucan endotransglucosylase/hydrolase (XTH)-specific activity decreased drastically during the infection process in both apple and tomato fruits. We suggest that XTH reduction during the infection might be related with the fungus attack mechanism. We also suggest that the decrease in activity and the consequent lower xyloglucan endotransglucosylation, together with the increase in endoglucanases, would permit fungal access to the cellulose−xyloglucan network, increase the efficiency of cellulose hydrolysis, and thus facilitate the progress of the fungal infection. The results confirm the importance of hemicellulose degradation in the breakdown of plant cell walls, causing cell-wall loosening, increasing the porosity of the wall, and allowing the colonization of plant tissue.

KEYWORDS: Cell wall; Cellulases; fruit infection; hemicelluloses; *Lycopersicum esculentum*; *Malus domestica*; *Penicillium expansum*; xyloglucan endotransglucosylase/hydrolase

INTRODUCTION

Penicillium expansum is the causal agent of blue mould rot, the most devastating pathogen in harvested apples (*1*). Apples are an important worldwide crop that constitute one of the most commercially important temperate fruits that can be stored for many months, but significant losses of apple fruit in storage are suffered by the blue mould rot produced by *Penicillium expansum*. Most pathogenic species of *Penicillium* have a limited host range, suggesting unique adaptations to a particular host, while *P. expansum* has a broad host range. It is well-known that plants protect themselves from attack, and in this case, the plant cell wall is the major barrier that protects the cell from pathogen infections. Most phytopathogenic microorganisms produce a battery of enzymes that are capable of degrading the different cell-wall polysaccharides, such as pectins and hemicelluloses (*2*−*4*), and allow the fungus to attack the cell. Of the multiple enzymes involved in the degradation of different cell-wall components, those breaking down pectic polymers have undergone the most intensive investigation. This is because the pectinases are typically produced first, in the largest amounts, and are the only cell-wall-degrading enzymes capable of macerating plant tissue and killing plant cells on their own (*5*). The activation of pectin cell-wall-degrading enzymes during fungal infection is considered an important pathogenicity factor, and a significant amount of work has been published. Direct evidence exists of the importance of polygalacturonase (*6*−*8*), and pectin lyases (*9*, *10*) in pathogenicity, which has been reported.

In addition to the degradation of pectic polymers, degradation-associated changes in hemicellulosic polysaccharides are likely to be important in the breakdown of plant cell walls and the colonization of plant tissue (*11*). A breakdown of xyloglucan could weaken the wall and provide access to fungal cellulases to degrade the cellulose microfibrills. Also, side chains of hemicellulosic polysaccharides might be degraded by glycosidase, facilitating access for endo forms of cell-wall-degrading enzymes (*12*). Also, there are certain enzymes related with the hemicellulosic turnover and metabolism such as xyloglucan endotransglucosylase/hydrolase (XTH) that appear to be involved in cell expansion as well as developmental processes that involve cell-wall degradation, such as in fruit ripening (*13*). However, if XTH has a role during the cell-wall disassembly that occurs during the fungal infection process, it has yet to be investigated.

To further investigate the mechanism of fungal infection, we characterized the changes in cell-wall hemicellulosic polysaccharides and the hemicellulose-degrading enzymes associated with fruit infection in two different fruits, apple and tomato. Both fruits have a different cell-wall structure and composition

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(*14*) and also differ in the specificity of the pathogen. Apple fruit is the usual host of *P. expansum*, although it also infects other fruits such as tomato. We particularly wanted to evaluate the implication of the plant, fungal β-glucanases, and XTH during the fruit infection to further characterize the role of the hemicellulosic hydrolytic enzymes in the cell-wall degradation process. We were aware that in control fruits, the enzymes studied come from the plant, while in the infected fruits, we were measuring enzymatic activities of both plant and fungal origin together. Additional experiments trying to determine the possible origin (plant or fungal) were made to establish the relationship between the changes in cell-wall structure and the hemicellulose-degrading enzymes activated during infection, to clarify the mechanisms involved in the cell-wall degradation process.

MATERIALS AND METHODS

Plant Material. Apple fruits (*Malus domestica* var. Golden) and tomato fruits (*Lycopersicum esculentum* var. canario) were purchased at the local market from September to November (2000−2002). Fruits were selected based on being of a similar size and at a similar stage of ripening.

***P. expansum* Growth and Fruit Infection.** *P. expansum* was isolated from a diseased Golden Delicious apple and maintained as a single-spore culture on potato dextrose agar (PDA, Difco, Laboratories) at 20 °C. For fruit inoculation, spores from 7-day-old cultures were suspended in sterile distilled water and filtered through glass wool. A total of 10 μL of the conidial suspension, containing 2×10^6 spores mL^{-1}, was used for the infection experiments. Fruits were infected on two opposite sides by injection with a microsyringe. Control fruits were inoculated with 10 μL of distilled water. In addition, in some experiments, fruits were autoclaved at 121 °C for 1 h before inoculation. Fruits were then held at 24 °C and at 60% relative humidity during 3 days. After the incubation time, cylindrical samples of 1 cm diameter and 1 cm long were obtained from around the injection point of the fruits. Samples were frozen at −20 °C until the experiments were carried out.

Preparation and Analysis of the Fruit Cell Walls. Fruit samples were thawed and peeled, and the pericarp (100 g) was homogenized in 300 mL of 5 mM sodium metabisulfite for 2 min at 4 °C with an Omnimixer (Sorval). The homogenate was centrifuged at 8500*g* for 5 min at 4 °C, and then the pellet was washed twice with 96% ethanol and centrifuged (8500*g* for 5 min). The alcohol-insoluble residue was suspended in 96% ethanol, boiled for 30 min, washed twice with ethanol, and stored overnight at 4 °C in ethanol. The followed day, the suspension was centrifuged (8500*g* for 5 min), and the pellet was washed twice with acetone, followed by methanol/chloroform (1:1, v/v), and finally air-dried to a constant dry weight.

Extraction of Hemicellulosic Polysaccharides. Sequential extraction of the cell-wall material was undertaken as described by Cutillas-Iturralde et al. (*15*, *16*). Pectins were extracted with 0.05 M *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid and 0.05 M Na_2CO_3, and the depectinated cell-wall material was used for the subsequent extraction of hemicellulosic polysaccharides. The residue was extracted with 4% KOH containing 20 mM $NaBH_4$ under a nitrogen atmosphere for 24 h and centrifuged at 30000*g* for 10 min. The sediment was then re-extracted for another 2 periods under the same conditions. The remaining residue then extracted 3 times for 24 h with 24% KOH containing 20 mM $NaBH_4$, under a nitrogen atmosphere. In both the 4 and 24% extractions, the 3 extracts were combined, neutralized with acetic acid, extensively dialyzed against distilled water, concentrated with a rotary evaporator, and freeze-dried. These 2 hemicellulosic fractions are referred to as 4 and 24% KOH, respectively.

Hemicelluloses Analysis. Lyophilized powders (5.0 mg) of the hemicellulosic fractions were hydrolyzed with 2 M trifluoroacetic acid at 121 °C for 1 h, and the content of the total sugars was determined from the supernatant using the phenol sulfuric acid method (*17*). The xyloglucan content was determined from the hemicellulosic fractions using the iodine-staining method (*18*).

Gel chromatography of each fraction was carried out on a Sepharose CL-4B column (115 × 1.4 cm) equilibrated with 1 M NaOH. The columns had been calibrated with authentic dextrans (9.4, 70, 510, and 5000 kDa) purchased from Pharmacia and Sigma Chemical Co. The samples (ca 8 mg) were dissolved in 2 mL of 1 M NaOH and eluted with 1 M NaOH at a flow rate of 19 mL h^{-1}. A total of 2 mL fractions were collected, and an aliquot of each fraction was assayed for total sugars and xyloglucan using the phenol−sulfuric acid method for total sugars (*17*) and the iodine-staining method (*18*) for xyloglucan determination. The molecular mass of the hemicellulosic fractions and of xyloglucan was estimated from the respective gel-permeation chromatogram using the formula $M = \sum(M_i \times W_i)/\sum W_i$, where M is molecular mass, W_i is total sugar or xyloglucan content of the ith fraction, and M_i is molecular mass of the ith fraction estimated from the calibration curves (*19*).

Protein Extraction. Both soluble and ionically bound proteins were extracted together by homogenization of partially thawed apple or tomato fruits pericarp (0.5 g/mL) in 40 mM sodium acetate at pH 5.0, containing 13 mM CDTA, 10 mM 2-mercaptoethanol, 1% (w/v) poly(vinylpyrrolidone) 10, and 1 M NaCl. The tissue was homogenized for 1 min in an Omnimixer (Sorvall) set at half-maximum speed. The homogenate was extracted with stirring for 18 h and then centrifuged at 35000*g* for 15 min. The supernatant was filtered through a glass fiber filter, and proteins were precipitated by the addition of solid ammonium sulfate to a final concentration of 80% (w/v). The precipitated proteins were collected by centrifugation at 35000*g* for 15 min, and the pellet was dissolved in 40 mM sodium acetate at pH 5.0 and desalted on a Sephadex G-25 column (1.4 × 19.0 cm), which had been equilibrated with the same buffer. All extraction procedures were carried out at 4 °C. The protein content of the extracts was assayed by the Coomassie Blue G dye-binding method (*20*). In some experiments, infected and control samples of apple fruit were pooled and the proteins were extracted as mentioned above.

Enzyme Assays: β(1−4) Glucanase. Glucanase activity was assayed viscosimetrically. The substrate was 0.3 mL of 1.2% (w/v) carboxy methyl (CM)-cellulose (sodium salt, high viscosity, Sigma Chemical Co.) or 10% (w/v) tamarind xyloglucan in 40 mM sodium acetate at pH 6.0. Reaction mixtures containing 0.9 mL of 1.2% CM-cellulose (sodium salt, high viscosity, Sigma Chemical Co.) or 10% tamarind xyloglucan and 200 μL of crude enzyme (0.5−0.6 mg protein/mL) in 40 mM sodium acetate at pH 5.0 were incubated for different periods of time at 37 °C. At intervals, the solution was sucked into a 0.2 mL pipet, the efflux time was determined, and the relative viscosity was calculated.

Xyloglucan Endotransglucosylase. Xyloglucan endotransglucosylase was assayed as reported by Fry et al. (*21*), and [^{3}H]XXXGol was used as the acceptor for the endotransglucosylation reaction. Xyloglucan heptasaccharide XXXG was prepared as described previously (*22*). [^{3}H]XXXGol was prepared by reduction of the reducing terminal glucose moiety of nonradioactive XXXG with NaB_3H_4. The solution of [^{3}H]XXXGol used for the XTH assays had a specific activity of 22.5 TBq mol^{-1}. Reaction mixtures (total volume of 40 μL) containing 5 mg mL^{-1} of partially purified apple xyloglucan, 0.85 kBq [^{3}H]XXXGol, 50 mM MES (Na^+) at pH 6.0, and 25 μL of enzymatic extract (0.5−0.6 mg mL^{-1}) were incubated for 1 h at 25 °C. The reaction was stopped by the addition of 100 μL of 20% (w/v) formic acid, and the solution was then dried on 5 × 5 cm^2 Whatman 3MM filter paper, washed for 30 min in running tap water to remove unchanged [^{3}H]XXXGol, re-dried, and assayed for ^{3}H by scintillation counting. Inactivated controls were carried out in the same way using enzyme previously boiled for 30 min.

Infection Experiments and Analysis. Three independent fruit infection experiments were performed, using 20 fruits in each experiment. Gel-permeation chromatography was performed twice. All enzyme assays were performed 3 times. Values shown on graphs and tables are the means of the 3 independent experiments and their replicas, using 20 fruits in each experiment ±standard error (SE).

Table 1. Sugar Content of Hemicelluloses and Xyloglucan (% Cell-Wall Dry Weight) Extracted from Control and Infected Apple and Tomato Fruits[a]

		apple fruit (% dry weight)		tomato fruit (% dry weight)	
		control	infected	control	infected
4% KOH	total sugars	5.1 ± 0.8	9.1 ± 1.1	7.1 ± 2.6	9.0 ± 2.6
	xyloglucan	1.6 ± 0.1	3.1 ± 0.7	1.0 ± 0.0	1.1 ± 0.2
24% KOH	total sugars	21.7 ± 3.2	13.0 ± 2.3	10.43 ± 0.2	9.8 ± 1.3
	xyloglucan	10.6 ± 0.2	5.7 ± 0.8	5.9 ± 0.3	5.3 ± 0.4

[a] Hemicelluloses were extracted with 4 and 24% KOH.

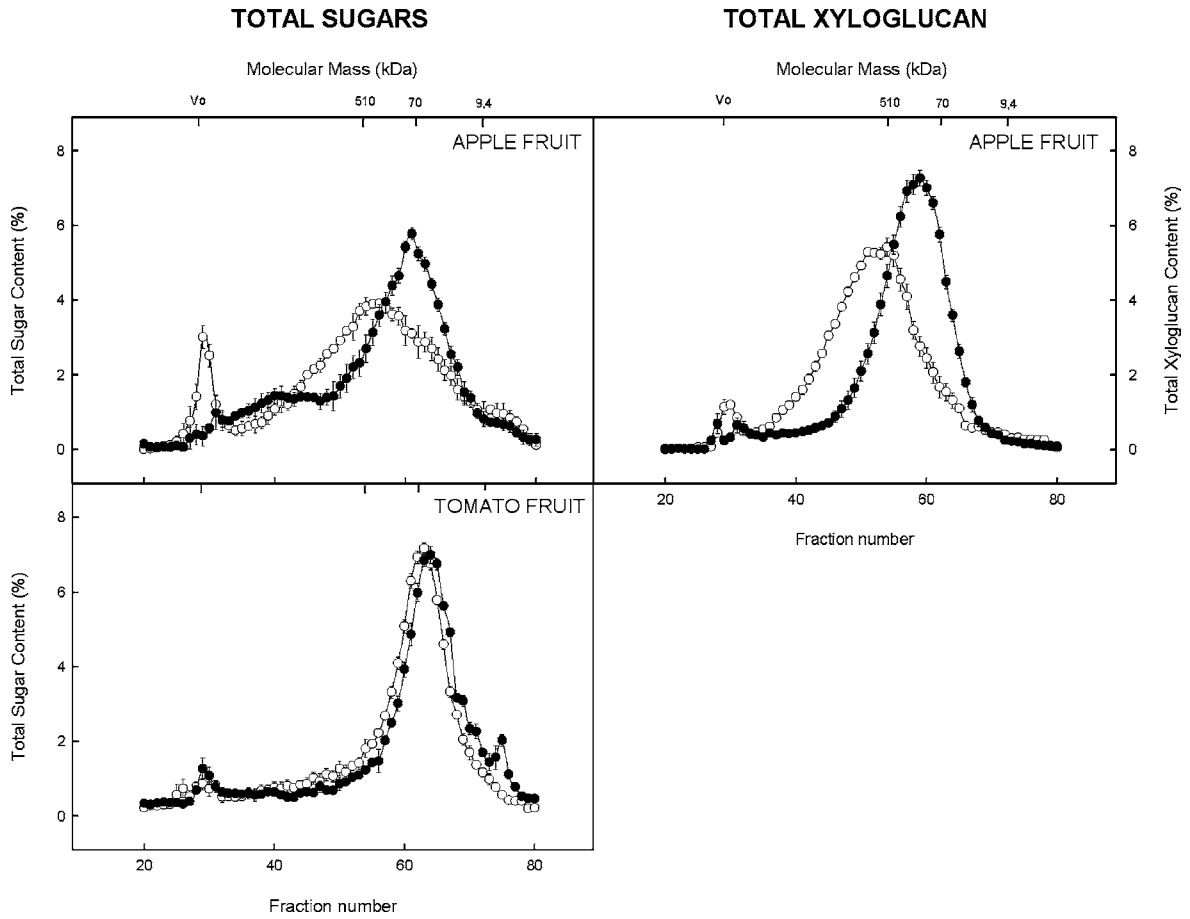

Figure 1. Distribution of molecular mass of total sugars and xyloglucan of hemicelluloses extracted with 4% KOH and chromatographed on a Sepharose CL-4B column. Fractions were extracted from the control (○) and infected (●) apple and tomato fruits. Calibration scale, obtained using authentic dextrans (kDa), is shown at the top (Vo, void volume).

RESULTS

We investigated the changes in the composition of the fruit cell wall and in the molecular mass of hemicellulosic fractions, during the infection of apple and tomato fruits by *P. expansum*.

The total sugar content in soluble hemicelluloses is shown in **Table 1**. Hemicelluloses were extracted sequentially with 4 and 24% KOH, representing about 27 and 19% of the cell-wall polysaccharides in apple and tomato fruits, respectively. Xyloglucan was present in both fractions but mainly extracted with 24% KOH, the main hemicellulosic fraction. In this fraction, we observed a decrease in total sugars and xyloglucan in infected fruits, with this decrease being more notable in apple fruits.

The results of the gel-permeation chromatography on a Sepharose CL-4B column of the hemicellulosic polysaccharides extracted with 4 and 24% KOH are shown in **Figures 1** and **2**. In the hemicelluloses extracted with 4% KOH from control apple fruits (**Figure 1**), two peaks of total polysaccharides appeared on the chromatograms, the first in the void-volume region and the second in the fractionation range of the column. In the infected fruits, we observed that the first peak was absent and there was a shift of total sugars in the second peak toward a lower molecular mass region. The xyloglucan distribution was similar to that of the total polysaccharides in control apple fruits and also showed an important shift toward a lower molecular mass region in infected fruits. However, when we look at tomato fruit, most of the sugars were eluted in a single and narrower peak located in the 70-kDa region and again, in the infected fruits, a shift to a lower molecular mass region was observed. The xyloglucan content was too low in this fraction to determine the elution profile. The fraction of hemicelluloses extracted with 24% KOH (**Figure 2**) showed a total sugars distribution with a broad peak, with emphasis on a shift toward a lower molecular mass region in the infected fruits. Xyloglucan eluted in the same fractionation range as total sugars, and a depolymerization of this polysaccharide was observed during infection. In control tomato fruits, two peaks were observed in this fraction, within the fractionation range of the column. The first peak decreased in the infected fruits, appearing as a shoulder of the second one

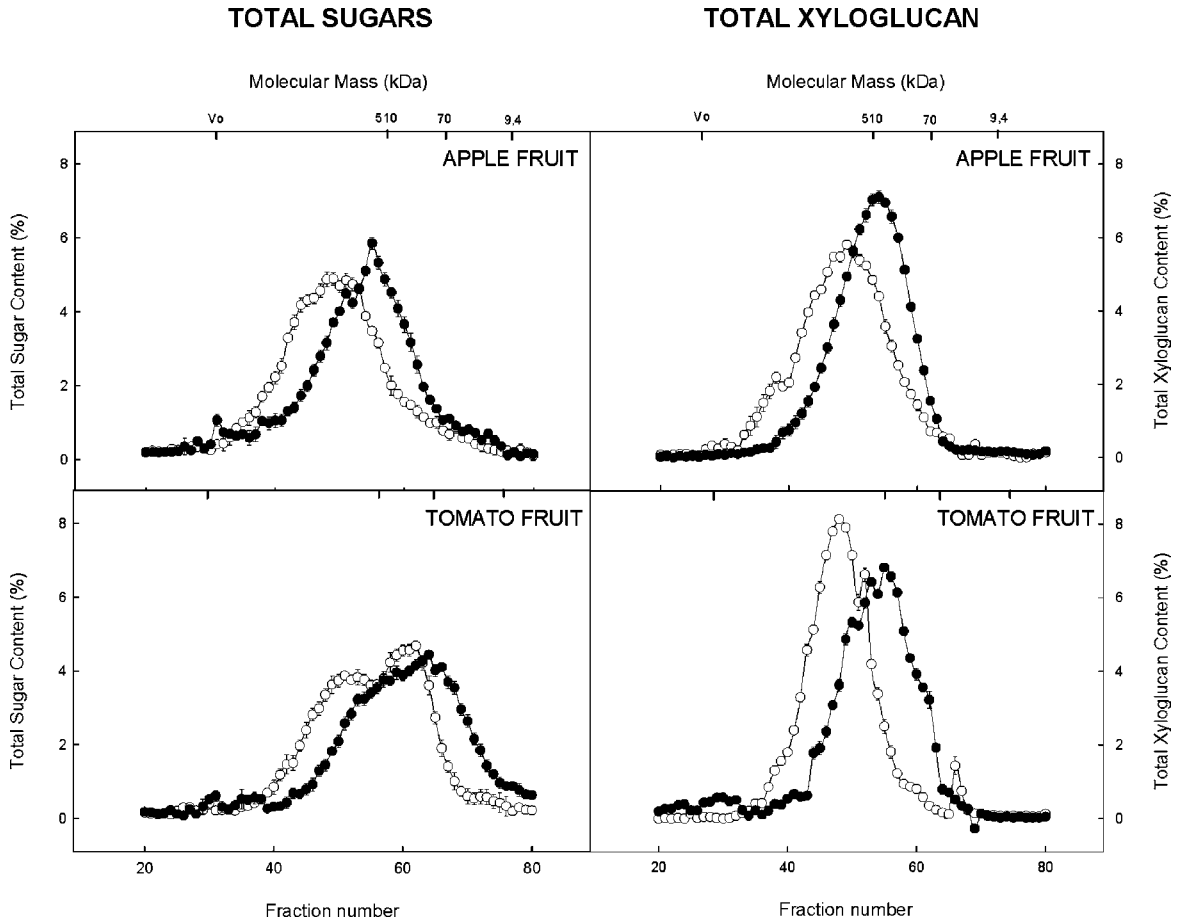

Figure 2. Distribution of molecular mass of total sugars and xyloglucan of hemicelluloses extracted with 24% KOH and chromatographed on a Sepharose CL-4B column. Fractions were extracted from the control (○) and infected (●) apple and tomato fruits. Calibration scale, obtained using authentic dextrans (kDa), is shown at the top (Vo, void volume).

Table 2. Changes in the Average Molecular Mass of Total Sugars and Xyloglucan of Hemicelluloses Extracted from Control and Infected Apple and Tomato Fruits[a]

		apple fruit molecular mass (kDa)		tomato fruit molecular mass (kDa)	
		control	infected	control	infected
4% KOH	total sugars	1029 ± 68	870 ± 42	741 ± 23	716 ± 11
	xyloglucan	954 ± 47	487 ± 34	nd[b]	nd[b]
24% KOH	total sugars	1331 ± 31	919 ± 27	661 ± 26	473 ± 10
	xyloglucan	1300 ± 26	412 ± 19	1133 ± 35	685 ± 14

[a] Average masses were calculated from the values plotted in **Figures 1** and **2**. [b] nd = not detected.

and eluting in a lower molecular mass region. Xyloglucan was present in this fraction and with a different distribution from that of the total polysaccharides, because it eluted only in the first peak. Again, as the infection progressed, a shift toward the lower molecular mass region was observed.

The average molecular masses of total sugars and xyloglucan in both hemicellulosic fractions are shown in **Table 2**. In apple fruit, we observed an important reduction in the molecular mass of total sugars in infected fruits, compared to that in control fruits. This reduction was observed in both hemicellulosic fractions (15 and 31%, at 4 and 24%, respectively). When we look at xyloglucan, the reduction in molecular mass during infection was even higher (49 and 68%, at 4 and 24%, respectively). However, in tomato fruits, in the hemicelluloses extracted with 4% KOH, only a slight decrease in the molecular mass of total sugars was observed during infection, with the most dramatic changes being in the hemicelluloses extracted with 24% KOH. In this last fraction, an important reduction in the molecular mass of total sugars and xyloglucan between infected and control fruits was found (28 and 39%, respectively).

We also measured the activity of hemicelluloses degrading cell-wall enzymes, during the infection of apple and tomato fruits by *P. expansum*. We were aware that, in control fruits, the enzymes studied come from the plant, although, in the infected fruits, however, we were measuring enzymatic activities with both plant and fungal origin. $\beta(1-4)$ Glucanase activity was determined by a decrease in the viscosity of CM-cellulose and tamarind xyloglucan (**Figure 3**). Apple fruit protein extract produced a moderate decrease in the viscosity of both substrates, with the decrease being dramatically faster when enzyme extract from infected fruits was used. We found similar results with the tomato protein extract, although specific activity was much lower than in apple fruit. In any case, the results suggest the importance of this xyloglucan-degrading enzyme during the infection and fruit colonization. In addition to $\beta(1-4)$ glucanase, XTH activity, also implicated in xyloglucan metabolism, was

Table 3. Specific Activity of Xyloglucan Endotransglucosylase from Apple and Tomato Fruits[a]

	^{3}H-XXXGol incorporation (Bq KBq^{-1} μg of protein^{-1} h^{-1})	
	apple fruit	tomato fruit
control fruits	3.06 ± 0.13	1.31 ± 0.05
infected fruits	0.77 ± 0.03	0.19 ± 0.02
previously autoclaved infected fruits	nd[b]	nd[b]
control and infected protein extracts pooled	1.76 ± 0.10	0.86 ± 0.06
control and infected fruits pooled	1.21 ± 0.10	nd[c]

[a] The protein extract to measure the XTH activity was obtained from (a) previously autoclaved infected fruits, (b) equal amounts of the protein extract of control and infected fruits that were pooled, and (c) infected and control samples of apple fruit that were pooled for each fruit and then the proteins were extracted. Specific activity of XTH from control and infected fruits from **Figure 5** are also included for comparison. Values shown are the means of 3 independent experiments, using 20 fruits in each experiment ±SE. [b] nd = not detected. [c] nd = not determined.

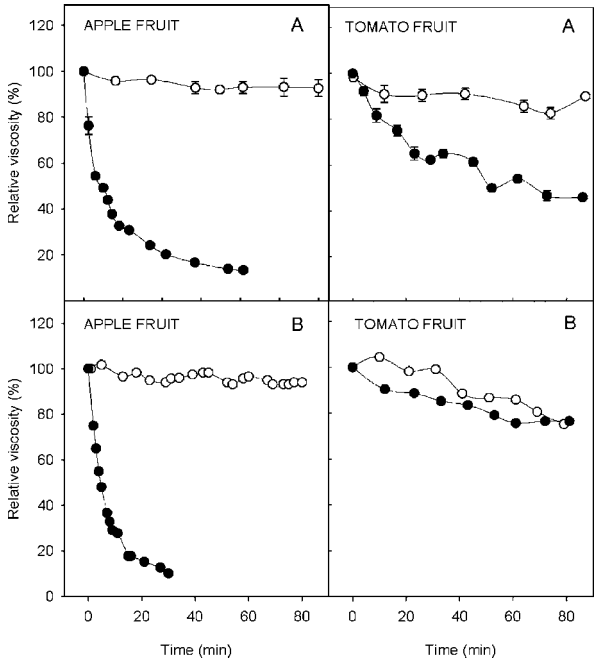

Figure 3. Kinetics of the decrease in viscosity of (A) CM-cellulose or (B) tamarind xyloglucan, during incubation with protein extracts of apple and tomato fruit. (○) Control fruits. (●) Infected fruits. The viscosimeter flow time at time 0, which was 50 s, was arbitrarily set to a relative viscosity of 100%. Controls with boiled enzyme did not show a decrease in viscosity with incubation time (not shown). Values shown are the means of 3 independent experiments, using 20 fruits in each experiment ±SE.

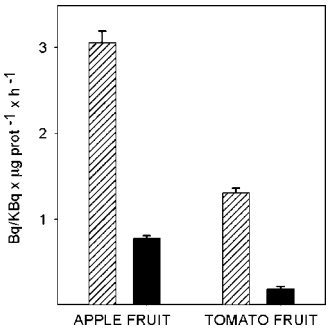

Figure 4. Specific activity of xyloglucan endotransglucosylase from apple and tomato fruit. Dashed bars indicate control fruits. Filled bars indicate infected fruits. Values shown are the means of 3 independent experiments, using 20 fruits in each experiment ±SE.

determined (**Figure 4**). The results showed the presence of soluble XTH activity in both apple and tomato fruits, but during the infection, an important decrease in XTH-specific activity occurs in both fruits.

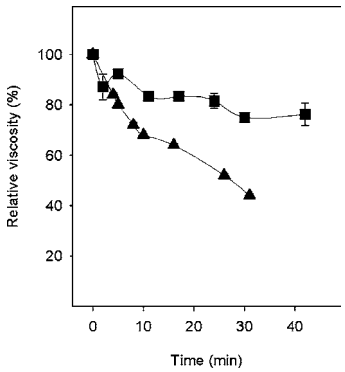

Figure 5. Kinetics of the decrease in viscosity of CM-cellulose during incubation with protein extracts of autoclaved apple (▲) and tomato (■) fruits. The viscosimeter flow time at time 0, which was 50 s, was arbitrarily set to a relative viscosity of 100%. Controls with boiled enzyme did not show a decrease in viscosity with incubation time (not shown). Values shown are the means of 3 independent experiments, using 20 fruits in each experiment ±SE.

Additional experiments were made to try to determine the possible origin (plant or fungal) of the reduction in XTH activity. Apple and tomato fruits were autoclaved (to inactivate all of the fruit enzymatic activities), and the fruits were subsequently infected with *P. expansum*. Fungus growth and infection progress was observed as well as fungal production of $\beta(1-4)$ glucanase (using CMC as the substrate) as shown in **Figure 5**. However, no fungal XTH activity was detected after infection in previously autoclaved apple and tomato fruits (**Table 3**). Additionally, equal amounts of the protein extracts from control and infected fruits were pooled after having being individually extracted from and then assayed for XTH activity. Values of XTH-specific activity were proportional to the respective controls, indicating that no XTH inhibitor was present in the protein extract from the infected fruits. Similar results on XTH activity were obtained when infected and control samples of apple fruit were pooled, the proteins were extracted, and the XTH was assayed.

DISCUSSION

In this work, we wanted to further study the plant−microbe interaction during fruit fungal infection, at a plant cell-wall structural level. We wanted to determine if degradation-associated changes in hemicellulosic polysaccharides are important determinants in the breakdown of plant cell walls and the colonization of plant tissue, as has previously been suggested (*11*).

To explore this possibility, we infected apple and tomato fruits with *P. expansum* and we characterized the infection-associated modifications of hemicelluloses structure as well as the hydro-

lytic enzymes produced during the fungus infection. We found that, in both apple and tomato fruits, the hemicellulosic polysaccharides and xyloglucan were solubilized from the cell wall during infection (**Table 1**). At the same time, we observed a reduction in the molecular mass of hemicelluloses in infected fruits compared to the control fruits, with this reduction being particularly important in xyloglucan (**Figures 1** and **2** and **Table 2**). This polysaccharide reduction in the molecular mass was more noticeable in apple fruit; therefore, we suggest that this difference between apple and tomato fruits is maybe due to the fact that apple fruit is the usual host of *P. expansum*, although it also infects other fruits such as tomato. Because we found such a large change in the xyloglucan structure during fungal infection, we have also investigated the increase of xyloglucan-degrading enzymes in apple and tomato fruits during infection by *P. expansum.* As expected, our results indicated that during infection in both apple and tomato fruits, an increase in β-glucanases, active against CMC and xyloglucan, occurred (**Figure 3**). In control fruits, the origin of the β-glucanases is obviously the fruit; however, in the infected fruits, we were measuring both the fruit and the fungus β-glucanases together. Our experiments of fungal infection with autoclaved fruits showed an increase of $\beta(1-4)$ glucanase during infection (**Figure 5**); therefore, we can safely assume that the increase in activity of this enzyme during infection had a fungal origin. However, when we compare the specific activity between both fruits, we found that the $\beta(1-4)$ glucanase activity was much higher in apple than in tomato fruits. It could appear that the differences can be attributed to different reasons. On one hand, xyloglucan structure is different in both fruits: in apples, the branching pattern is type XXXG, whereas in tomato, the branching pattern is type XXGG (*14*). On the other hand, previously mentioned, there are also differences in the specificity of the pathogen, with apple fruit being the usual host of *P. expansum*. This last possibility is in agreement with the suggestion of a role of plant cell-wall composition and structure in disease resistance (*23*).

Another enzyme that has reportedly been involved in the modification of the cellulose−xyloglucan network, acting cooperatively with endoglucanases and glycosidases, is XTH (*13*). We found this enzyme present in both apple and tomato fruits; however, our results showed that the activity decreased drastically during the infection process in both fruits (**Figure 4**). One possibility that might explain such a reduction could be that the decrease in XTH activity could be due to proteolitic breakdown of endogenous enzymes by the invading pathogen. It is obviously a possibility; therefore, we did an experiment to further explore this avenue. We measured XTH activity of pooled equal amounts of the protein extracts obtained from control and infected fruits, first apple and then tomato. We also measured XTH activity, where proteins were extracted from pooled infected and control samples of apple fruits (**Figure 5** and **Table 3**). The results suggested that the decrease in XTH activity in the infected fruits was not a consequence of the presence of an XTH inhibitor in the infected fruit or in the protein extract and that the XTH activity was specifically reduced during infection. Our suggestion is that the decrease in XTH activity during infection could be specifically produced by the fungus. This XTH inhibition, in the context of the plant−microbe interaction, could be explained as follows: the decrease in XTH activity caused by the fungus reflects a lower xyloglucan endotransglucosylation that, together with the increase in endoglucanases, facilitates the colonization of the host cell wall. During infection, the increase in fungal β-glucanases increased the xyloglucan hydrolysis. This then lowered the degree of polymerization of xyloglucan and increased the concentration of soluble xyloglucan. In consequence, the donors for the transglucosylation reaction catalyzed by XTH increased, and the enzyme could again integrate xyloglucan into the cell wall. However, if the XTH activity is inhibited during infection, this possible restructuring role of XTH is not possible and the hemicellulose degradation continued. This suggestion is currently under further investigation.

To summarize, our results showed an important hemicellulosic and xyloglucan degradation associated with *P. expansum* infection and the activation of hemicellulosic cell-wall-degrading enzymes such as glucanases. The results confirm the importance of hemicelluloses degradation in the breakdown of plant cell walls causing cell-wall loosening, increasing the porosity of the wall, and allowing the colonization of plant tissue. In addition, the above findings provide evidence for the hypothesis that a decrease in XTH activity during infection could be related to an increase in the access to the microfibrill surface of the cellulase complex, increasing the efficiency of cellulose hydrolysis and facilitating the progress of the fungal infection.

ABBREVIATIONS

XTH, xyloglucan endotransglucosylase/hydrolase.

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