Enzymatic Degradation of Potato Cell Walls in Potato Virus X-Free and Potato Virus X-Infected Potato Tubers by Fusarium roseum 'Avenaceum'

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ABSTRACT

Enzyme preparations from culture filtrates of *Fusarium roseum* 'Avenaceum' or diseased tissue solubilized up to 54.9% of the noncellulosic wall polysaccharides in isolated potato cell walls when incubated together at 30 C for 5 hours at pH 7.0. Cell walls from potato virus X (PVX)-free tubers contained approximately 30% galactose, 26% glucose (cellulose), 16% galacturonic acid, 7% arabinose, 3% xylose, 3% glucuronic acid and 1% rhamnose. Tubers infected with PVX are more resistant to *F. roseum* 'Avenaceum' than PVX-free tubers. The polysaccharide composition of cell walls from PVX-infected tubers was comparable to that of walls from PVX-free tubers except for a decrease in galactose content. *F. roseum* 'Avenaceum' produced polysaccharidases in both PVX-infected and PVX-free tissue. Analysis of cell wall composition in PVX-free tubers infected with *F. roseum* 'Avenaceum' indicated that the noncellulosic cell wall polysaccharides were removed during pathogenesis. Cell walls from PVX-free and PVX-infected tubers were equally susceptible to enzymatic decomposition.

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Fusarium roseum (Lk.) Snyd. and Hans. 'Avenaceum' [*Fusarium avenaceum* (Fries) Sacc.] is a dry-rot pathogen of potato tubers (14, 22). Recently, it was demonstrated that potato tubers free of potato virus X (PVX) are more susceptible to dry-rot caused by this pathogen than are tubers infected with PVX (13, 14). Enzymes capable of degrading plant cell wall polysaccharides are considered to play a significant role in pathogenesis (2, 4, 5). *F. roseum* 'Avenaceum' is known to produce in culture as well as during pathogenesis the following polysaccharide degrading enzymes: exopolygalacturonase, endopolygalacturonate *trans*-eliminase, endo-β-1,4 galactanase, cellulase, xylanase and two arabanases. Isolated potato cell walls serve as an inducer for all of these enzymes (23, 25).

The objectives of this investigation were: (i) to examine the ability of the polysaccharide degrading enzymes produced by *F. roseum* 'Avenaceum' to degrade potato tuber cell walls, (ii) compare the carbohydrate compositions and susceptibilities of potato cell walls isolated from PVX-free and PVX-infected potato tubers to enzymatic decomposition, (iii) examine cell wall breakdown in potato tubers during pathogenesis by *F. roseum* 'Avenaceum', and (iv) determine if polysaccharide degrading enzymes are produced by *F. roseum* 'Avenaceum' in PVX-infected tubers.

MATERIALS AND METHODS.—A virulent isolate of *F. roseum* 'Avenaceum' was cultured from a potato tuber exhibiting dry-rot symptoms. This isolate was maintained as previously described (23) and used throughout this investigation.

Sources of polysaccharide degrading enzymes.—Filtrates of *F. roseum* 'Avenaceum' grown on a mineral salts liquid medium supplemented with 0.5% sodium polypectate (Sunkist Growers, Inc.), lupin galactan [prepared by the method of Jones and Tanaka (15)] araban (Koch-Light Laboratories, Ltd.), or xylan (Nutritional Biochemicals Corp.) further purified by the procedure of Adams (1), were prepared (23). Also, filtrates from 10-day-old cultures of *F. roseum* 'Avenaceum', grown in the dark at 22 C in still culture, supplemented with 1.5% isolated potato cell walls (including 40% starch by weight) were used as an enzyme source. The polysaccharide-degrading enzyme activities in dialyzed culture filtrates were determined. The pH optima for exopolygalacturnase, endopolygalacturonate *trans*-eliminase, endo-β-1,4 galactanase, arabanase$_1$, arabanase$_2$, xylanase, and cellulase were approximately 5, 9, 5, 5, 9, 6, and 5, respectively. A unit of activity for a given enzyme represents that amount of enzyme which releases 1 μmole of product (reducing groups) from its substrate at its pH optimum at 30 C in 1 hour. Enzymes activities are given as units per mg protein.

Extracts of *F. roseum* 'Avenaceum'-infected potato tubers were also used as an enzyme source. PVX-free and PVX-infected tubers of the Katahdin variety (150-250 gm in weight) were inoculated 4-6 days after harvest, held for 2 weeks at 20 C, and then stored for 5 months at 4 C as previously described (25). Wounded tubers, injected with water rather than a spore suspension, were used as controls. After the storage period, necrotic tissue from the *F. roseum* 'Avenaceum' infected tubers and healthy tissue from the controls were extracted immediately with water or 200 mM 2-mercaptoethanol or stored 10 months at −10 C prior to extraction with water (w/v) as previously described (25). The frozen control tissue was extracted after only 2 months of storage at −10 C. Enzyme activities in dialyzed tissue extracts were measured in the same manner and expressed in the same units as the activities in culture filtrates.

Enzyme assays.—The activities of exopolygalacturonase, endopolygalacturonate *trans*-eliminase, endo-β-1,4 galactanase, arabanase$_1$, arabanase$_2$, xylanase, and cellulase were measured as previously described (25).

Protein determination.—Protein concentrations in dialyzed enzyme preparations were estimated by the method of Lowry et al. (21). Crystalline bovine serum

TABLE 1. Polymeric carbohydrate composition of potato cell walls isolated from potato virus X-free tubers and potato virus X-infected tubers

Potato Tuber Source	Constituents[a]						
	Gal[b]	*Xyl*	*Ara*	*Rha*	*Gal A*	*Glu A*	*Cell*
Virus X-free	29.5	2.8	7.5	1.4	16.3	3.3	25.7
Virus X-infected	23.0	2.4	6.6	1.3	16.7	3.4	25.6

[a]Constituents are given as mg of a component per 100 mg of cell wall (mg %).

[b]Abbreviations are galactose (Gal), xylose (Xyl), arabinose (Ara), rhamnose (Rha), galacturonic acid (Gal A), glucuronic acid (Glu A), and cellulose (Cell).

albumin (Sigma Chemical Co.) was used as a standard.

Preparation of potato tuber cell walls.—Potato cell walls were isolated from PVX-free and PVX-infected Katahdin tubers (150-250 g) after 3-5 months of storage at 4 C. Cell walls were isolated using a modification of the procedure of Hoff and Castro (12) as described by Mullen and Bateman (25). Cell-wall preparations prepared by this method contained 25 to 75% starch by weight. The preparations used in this study contained between 25 and 50% starch. The amount of starch in a given wall preparation was determined by stirring a 30-mg sample in 10 ml of 90% dimethyl sulfoxide (Eastman Kodak Co.) as described by Leach and Schoch (20), washing four times with 20-ml aliquots of water, and then treating with 120 μg α-amylase (Calbiochem, Inc.) in 3.0 ml phosphate buffer (100 mM, pH 7.0) with stirring at 26 C for 1 hour (27). The dry weight of cell wall in a given preparation was corrected by subtracting out the starch component.

Potato cell wall polysaccharide composition.—The aldose and uronic acid compositions of potato tuber cell walls were determined using the procedures described by Jones and Albersheim (16). Partial hydrolysis of the polysaccharides was accomplished by treatment with 0.2 N trifluroacetic acid. This was followed by enzymolysis with a dialyzed 0.1% (w/v) solution of lyophilized *Sclerotium rolfsii* enzyme preparation in 10 mM sodium acetate (pH 4.5). Acetate derivatives of the monosaccharides released were prepared and assayed by gas chromatography. Potato cell-wall cellulose was estimated by the anthrone procedure as described by Updegraff (28).

The ability of enzymes produced by *F. roseum* 'Avenaceum' to degrade isolated potato cell walls was examined in reaction mixtures containing 10 mg of cell walls, 1.0 ml of phosphate buffer (100 mM, pH 7.0), and 1.0 ml of enzyme. Reaction mixtures were incubated for 5 hours at 30 C and then centrifuged (approximately 5,000 *g*) for 5 minutes. The uronic acids and aldoses in the wall residue as well as in the supernatant were determined separately using the procedures of Jones and Albersheim (16). The residues and supernatants of buffer treated walls were assayed as controls. Also, enzyme preparations were assayed for sugar constituents. Constituent sugars found in the supernatant of buffer treated walls plus sugars detected in enzyme preparations (without cell walls) were always minor, but these values were subtracted from the respective aldoses and uronic acids found in the supernatant of enzyme treated walls. Also, the cellulose content of buffer treated walls was measured and compared to the cellulose content of enzyme treated walls.

RESULTS.—*Polysaccharide composition of potato cell walls prepared from virus X-free and potato virus X-infected tubers.*—The major sugar constituents of the cell walls from the medulla of PVX-free tubers were

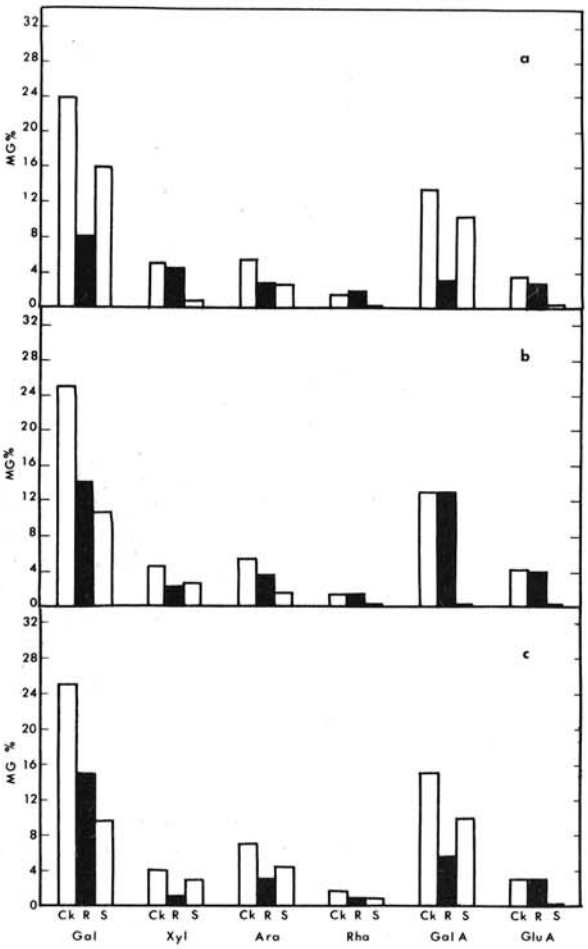

Fig. 1-(A to C). Degradation of isolated cell walls from potato virus X-free potato tubers by enzymes in culture filtrates of *Fusarium roseum* 'Avenaceum': **A)** filtrate from 8-day-old cultures grown at 22 C in a mineral salts medium supplemented with 0.5% sodium polypectate, **B)** filtrate from 8-day-old cultures grown at 22 C in a mineral salts medium supplemented with 0.5% xylan, and **C)**filtrate from 10-day-old cultures grown in a potato broth medium supplemented with 1.5% potato cell walls (containing 40% starch). Reaction mixtures contained 10 mg of cell walls, 1.0 ml of dialyzed culture filtrate, and 1.0 ml of 100 mM phosphate buffer (pH 7.0) in 0.01% Thimersal. After 5 hours of incubation at 30 C the wall residue (R) and the supernatant (S) were separated and analyzed for constituent sugars. Buffer treated walls (Ck) served as a control. Wall constituents are expressed as mg of a component per 100 mg of cell wall (MG %). Abbreviations are galactose (Gal), xylose (Xyl), arabinose (Ara), rhamnose (Rha), galacturonic acid (Gal A), and glucuronic acid (Glu A).

galactose, cellulosic glucose, galacturonic acid and arabinose. These constituents accounted for 79% of the cell wall (Table 1). Xylose, rhamnose, and glucuronic acid together accounted for 7.5% of the cell wall. The data obtained from the analysis of wall preparations from five different PVX-free tubers indicate that the potato cell wall contains at least 86.5% polysaccharide. Analysis of cell walls prepared from five potato tubers infected with PVX revealed that 79% of these walls could be accounted for as carbohydrate (Table 1). The relative amounts of constituent sugars in cell walls prepared from PVX-free and PVX-infected tissues were similar. Somewhat less galactose was recovered from PVX-infected tissue than from walls of PVX-free tissue (23% vs. 29.5%, respectively). Hoff and Castro (12) have reported that potato cell walls contain approximately 10% protein. If this value is assumed to be correct, about 95% of the potato cell wall composition can be accounted for by our analysis.

Enzymatic decomposition of isolated potato cell walls by enzymes produced by F. roseum 'Avenaceum'.—The culture filtrate of *F. roseum* 'Avenaceum' grown on a mineral salts medium supplemented with sodium polypectate contained endopolygalacturonate *trans*-eliminase (15 units/mg protein), arabanase$_1$ (2.5 units/mg protein), arabanase$_2$ (0.5 units/mg protein), endo-β-1,4 galactanase (5.6 units/mg protein), and xylanase (0.4 units/mg protein) (25) and was one of the most effective culture filtrates in solubilizing isolated potato cell walls. This preparation did not contain exopolygalacturonase or cellulase. After a 5-hour incubation at pH 7.0, 54.9% of the noncellulosic polysaccharides in the cell wall preparation were solubilized (Fig. 1-A). Filtrates from cultures grown on a salts medium supplemented with either galactan, araban or polygalacturonic acid contained enzymes that degrade α-1,4 galacturonosyl bonds, β-1,4 galactan, and araban (25). All of these preparations solubilized between 37.5 and 54.8% of the noncellulosic polysaccharides in cell walls prepared from PVX-free tubers during a 5-hour period. Filtrate from cultures supplemented with xylan contained arabanase$_1$ (0.3 units/mg protein), galactanase (0.7 units/mg protein), and xylanase (4.8 units/mg protein) and did not contain exopolygalacturonase, endopolygalacturonate *trans*-eliminase, arabanase$_2$, or cellulase (25). Filtrate from cultures supplemented with xylan solubilized only 27.9% of the noncellulosic polysaccharides in the potato cell wall preparation (Fig. 1-B). This filtrate did not solubilize the galacturonic acid and rhamnose components of the potato cell wall.

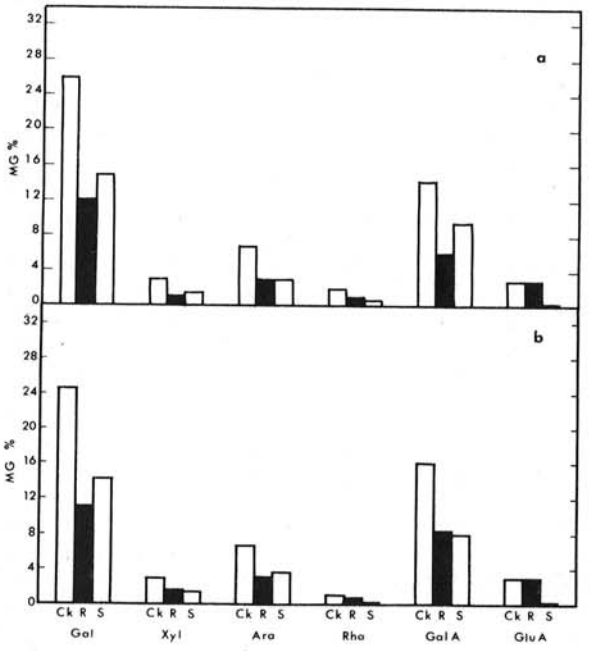

Fig. 2-(A, B). Degradation of isolated potato cell walls prepared from **A)** potato virus X-free and **B)** potato virus X-infected potato tubers by enzymes in a dialyzed water extract of *Fusarium roseum* 'Avenaceum'infected potato virus X-free tubers. The diseased tissue was frozen (−10 C) for 10 months prior to extraction. Reaction mixtures contained 10 mg of cell walls, 1.0 ml of extract (enzyme), and 1.0 ml of 100 mM phosphate buffer (pH 7.0) in 0.01% Thimersal. After 5.0 hours of incubation at 30 C, the wall residue (R) and supernatant (S) were separated and analyzed for constituent sugars. Cell walls incubated in buffer (Ck) served as a control. Wall constituents are expressed as mg of a component per 100 mg or cell wall (MG %). Abbreviations are galactose (Gal), Xylose (Xyl), arabinose (Ara), rhamnose (Rha), galacturonic acid (Gal A), and glucuronic acid (Glu A).

Isolated potato cell walls are good inducers of polysaccharide degrading enzymes by *F. roseum* 'Avenaceum'. Filtrates from 10-day-old cultures of the fungus grown on potato broth supplemented with potato cell walls contained exopolygalacturonase (2.3 units/mg protein), polygalacturonate *trans*-eliminase (1.0 unit/mg protein), arabanase$_1$ (2.9 units/mg protein), arabanase$_2$ (0.8 units/mg protein), galactanase (1.8 units/mg protein), xylanase (1.9 units/mg protein), and cellulase (1.3 units/mg protein) (25). This filtrate solubilized 54.8% of the noncellulosic sugars in the isolated cell wall preparation (Fig. 1-C). None of the culture filtrates tested solubilized significant amounts of glucuronic acid or cellulose in isolated potato cell walls.

The full complement of polysaccharide degrading enzymes produced by *F. roseum* 'Avenaceum' in culture has been demonstrated in PVX-free *Fusarium*-infected potato tubers (25). A water extract of *Fusarium*-infected potato tissue that had been frozen (−10 C) for 10 months prior to extraction to enhance enzyme recovery was used as an enzyme source to examine the relative susceptibilities of cell walls isolated from PVX-free and PVX-infected potato tubers to enzymatic degradation. This enzyme preparation contained exopolygalacturonase (5.0 units/mg protein), endopolygalacturonate *trans*-eliminase (7.9 units/mg protein), arabanase$_1$ (3.7 units/mg protein), arabanase$_2$ (3.7 units/mg protein), endo-β-1,4 galactanase (3.3 units/mg protein), xylanase (0.6 units/mg protein), and cellulase (3.9 units/mg protein). Cell walls from both sources were degraded to a comparable extent by this preparation (Fig. 2-A, B). After a 5-hour incubation at 30 C, 50% of the noncellulosic cell wall sugars of walls from PVX-free tubers and 48.6% of the same constituents from the PVX-infected tubers had been solubilized.

Enzymatic decomposition of potato cell walls during pathogenesis.—The occurrence of polysaccharide

TABLE 2. Polysaccharide degrading enzymes in extracts of potato virus X-infected potato tubers infected with *Fusarium roseum* 'Avenaceum'[a]

Enzyme	Enzyme activity (μmoles reducing groups/mg protein/hour)		
	Water extract (freshly harvested)	200 mM 2-mercapto-ethanol extract (freshly harvested)	Water extract (10 months at −10 C)
Galactanase	0.00	0.21	0.29
Xylanase	0.00	0.00	0.56
Arabanase$_1$	0.00	0.24	0.65
Arabanase$_2$	0.00	0.00	0.00
Exopolygalacturonase	0.00	0.00	0.81
Endopolygalacturonate *trans*-eliminase	0.00	0.00	0.26
Cellulase	0.00	0.00	0.00

[a]Diseased tissue from inoculated tubers stored at 4 C for 5 months was extracted (w/v) immediately after harvest with water or 200 mM 2-mercaptoethanol or frozen (−10 C) for 10 months prior to extraction with water. Extracts were dialyzed against distilled water at 4 C for approximately 20 hours. Reaction mixtures contained 0.8 ml of 0.1% substrate in 50 mM buffer and 0.2 ml of enzyme (extract). Buffer employed in a given assay was acetate buffer (pH 3.0 - 5.5), phosphate buffer (pH 5.5 - 7.0), or TRIS-HCl buffer (pH 7.0 - 9.0). Galactanase was assayed at pH 5.0, xylanase at pH 6.0, arabanase$_1$ at pH 5.0, arabanase$_2$ at pH 8.8, exopolygalacturonase at pH 5.0, endopolygalacturonate *trans*-eliminase at pH 8.5.

degrading enzymes in PVX-free *Fusarium*-infected potato tubers has been demonstrated. The recovery of these enzymes is greatly influenced by the extraction procedure employed. Water extracts of freshly harvested disease tissues contain relatively weak

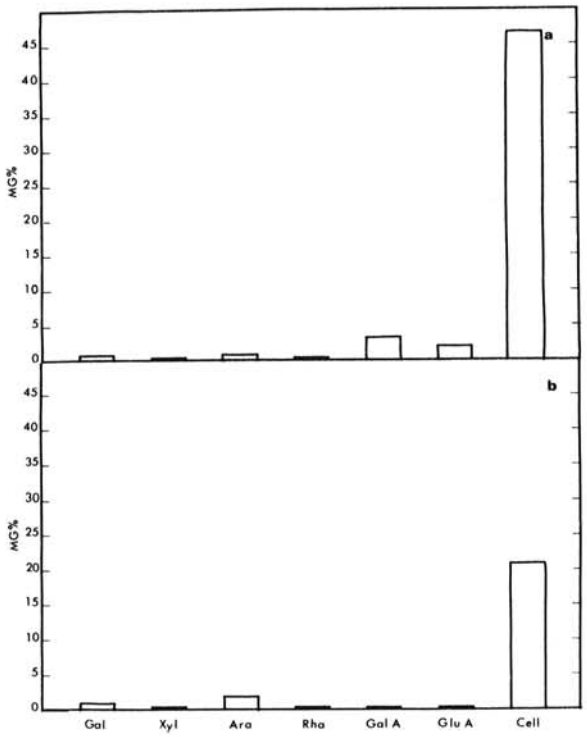

Fig. 3-(A, B). Recovery of carbohydrate constituents from isolated cell walls from **A)** potato virus X-free and **B)** potato virus X-infected potato tubers infected with *Fusarium roseum* 'Avenaceum'. Constituents are given as mg of a component recovered per 100 mg of cell wall (MG %). Abbreviations are galactose (Gal), xylose (Xyl), arabinose (Ara), rhamnose (Rha), galacturonic acid (Gal A), glucuronic acid (Glu A), and cellulose (Cell).

exopolygalacturonase, endopolygalacturonate *trans*-eliminase, arabanase$_1$, arabanase$_2$, galactanase, xylanase, and cellulase activities. Greatly enhanced activities of exopolygalacturonase, endopolygalacturonate *trans*-eliminase, galactanse, and xylanase were obtained when the extraction was carried out with 200 mM 2-mercaptoethanol. Also, if the disease tissue is frozen for 10 months prior to extraction with water, recoveries of the pectic enzymes, arabanases, galactanase and cellulase are enhanced over those obtained in water extracts of freshly harvested diseased tissue (25). Both of these treatments reduce polyphenol oxidase activity of the tissue extracts.

No polysaccharide degrading enzyme activities were detected in water extracts of freshly harvested PVX-infected tubers infected with *F. roseum* 'Avenaceum'. Tissue extracts prepared with 200 mM 2-mercaptoethanol contained only galactanase and arabanase$_1$ activities. Extracts of *Fusarium*-infected PVX-infected tuber tissues that had been frozen for 10 months prior to extraction contained endopolygalacturonase, endopolygalacturonate *trans*-eliminase, galactanase, arabanase$_1$ and xylanase activities (Table 2). The polysaccharide degrading enzymes are produced by *F. roseum* 'Avenaceum' in PVX-infected tubers during pathogenesis, but these enzymes are difficult to demonstrate in tissue extracts unless precautions are taken to prevent their inactivation.

Cell walls were prepared from both PVX-free and PVX-infected potato tubers infected with *F. roseum* 'Avenaceum' using the same procedure used to prepare walls from tissue free of *Fusarium*. These wall preparations were analyzed for cellulose (28) and noncellulosic polysaccharides (16). Wall preparations obtained from *Fusarium*-infected tissues were dark brown to black in color. The recovery of noncellulosic sugars from these preparations was poor (Fig. 3-A, B). This lack of recovery of these wall constituents may be due to their depletion by *F. roseum* 'Avenaceum' during pathogenesis and/or to failure of the procedures used in the analysis to hydrolyze and remove the noncellulosic polysaccharide constituents in these cell wall

preparations. The wall preparations from *Fusarium*-infected PVX-free tubers contained 47.5% cellulose and those from *Fusarium*-infected PVX-infected tissue contained 21% cellulose. About 30% of the cell wall preparation from the PVX-free *Fusarium*-infected and 65% of the cell wall preparation from the PVX-infected *Fusarium*-infected tissue were not accounted for as cellulose or other polysaccharides.

DISCUSSION.—*F. roseum* 'Avenaceum' is capable of producing enzymes that degrade the major polysaccharide constituents of plant cell walls (25). These enzymes include exopolygalacturonase, endopolygalacturonate *trans*-eliminase, two arabanases, endo-β-1,4 galactanase, xylanase, and cellulase. The pH optima for these enzymes range between pH 5 and 7 except for the endopolygalacturonate *trans*-eliminase and arabanase$_2$ which have pH optima near pH 9.0. The pH of water extracts of freshly harvested *Fusarium*-infected potato tuber tissue is about pH 7.3 (23, 25). Enzymes produced by *F. roseum* 'Avenaceum' in culture as well as those obtained from diseased tissue readily solubilize a substantial portion of the noncellulosic polysaccharides of isolated potato cell walls (Fig. 2).

In this study approximately 85% of the PVX-free Katahdin potato tuber cell wall was accounted for as polysaccharides. Based on our analysis, cell walls of the potato cultivar Katahdin contain approximately 30% galactose, 26% glucose (cellulose), 16% galacturonic acid, 7% arabinose, 3% xylose, 3% glucuronic acid, and 1% rhamnose. The protein content of potato tuber cell walls has been reported to be approximately 10% (12). The carbohydrate composition of the potato tuber cell walls reported in this paper is in general agreement with that reported for cell walls from the potato variety Superior (12). The potato cell wall is comprised primarily of pectic polysaccharides. The high galactose content of the potato cell wall appears to be a characteristic feature.

F. roseum 'Avenaceum', like *Rhizoctonia solani* (6), *Sclerotium rolfsii* (29) and other pathogens (2, 4, 5, 11, 19) that cause extensive decays of plant tissues, is well endowed with the ability to secrete a spectrum of enzymes capable of attacking plant cell wall polysaccharides. The endopolygalacturonate *trans*-eliminase produced by *F. roseum* 'Avenaceum' is capable of causing maceration of and cell death in potato tuber tissue (24). Maceration of tissue by this enzyme can be attributed to degradation of the polymeric galacturonides in the middle lamella of the cell wall. Based on current knowledge of primary cell wall structure (18), induction of cell death by this enzyme may be attributed to random degradation of the rhamnogalacturonan fraction in the primary wall which loosens this structure to the point that the cell protoplast ruptures due to osmotic stress (3). The significance of the other polysaccharide degrading enzymes in pathogenesis, aside from their potential effects on specifc cell wall polymers, remains to be determined. It appears that the pectic enzymes which split the α-1,4 bonds in the pectic fraction represent the key enzymes in plant cell wall degradation, and that the effect of other polysaccharide degrading enzymes on cell wall breakdown is enhanced after the walls are first attacked by pectic enzymes (3, 17, 27). Cellulose, as determined by the procedure of Updegraff (28), was not significantly broken down in potato cell walls incubated with the polysaccharidase complex, including cellulase, produced by *F. roseum* 'Avenaceum'. If the potato wall contains a xyloglucan of the type reported in the primary wall of *Acer pseudoplatanus* (7), the cellulase may contribute to cell wall degradation. Solubilization of wall components containing galactose, arabinose, xylose, and galacturonic acid by the complex of enzymes indicates that a number of the polysaccharidases produced by *F. roseum* 'Avenaceum' are capable of participating in cell wall decomposition during pathogenesis.

PVX-infected potato tubers are more resistant to *F. roseum* 'Avenaceum' than PVX-free tubers (13, 14). Analysis of cell walls prepared from the two types of tissue did not reveal any major differences in carbohydrate composition except for perhaps a 22% decrease in the galactose content of walls from virus-infected tissue (Table 1). Furthermore, wall preparations from PVX-infected and PVX-free tissues proved to be equally susceptible to degradation by a common enzyme preparation prepared from *F. roseum* 'Avenaceum'-infected potato tubers (Fig. 2).

We have observed that tissue extracts of PVX-infected tissue contain higher phenol oxidase levels than extracts of PVX-free tissues (Mullen and Bateman, *unpublished*). Also, the greater the polyphenol oxidase activity present in an extract of *F. roseum* 'Avenaceum'-infected tissue, the more difficult it was to demonstrate active polysaccharidases in these extracts. Oxidation of phenols in tissue extracts is known to inhibit plant cell wall degrading enzymes (9, 26). If precautions are taken to reduce or prevent phenol oxidase activity in tissue extracts, polysaccharide degrading enzymes produced by *F. roseum* 'Avenaceum' can be demonstrated in both PVX-free and PVX-infected potato tubers (25).

It appears that resistance of PVX-infected potato tubers to *F. roseum* 'Avenaceum' cannot be attributed to cell wall polysaccharide composition per se or to a lack of production of cell wall degrading enzymes in the resistant tissue by *F. roseum* 'Avenaceum'. Suberin and lignin-like substances are deposited in potato cell walls during pathogenesis (10) and cell walls prepared from both PVX-free and PVX-infected tubers infected with *F. roseum* 'Avenaceum' were quite dark in appearance. It may be that the cell walls in PVX-infected tissue, which contains a higher phenol oxidase level than virus-free tissue, become more rapidly melanized and thus more resistant to polysaccharide degrading enzymes than cell walls in PVX-free tubers.

Analysis of cell walls from *F. roseum* 'Avenaceum'-infected tubers by the procedures used in this study are difficult to interpret. It was obvious that considerable wall residues remained after their hydrolysis with trifluroacetic acid and enzymolysis with *S. rolfsii* enzymes. A good portion of these walls could not be account for as polysaccharide or as cellulose. It is realized that oxidized phenolics and other substances were deposited in these cell walls and wall fragments, and that these compounds may mask the polysaccharides and render them resistant to the hydrolytic steps in the analytical procedure. In spite of these difficulties, the analysis of cell walls from *Fusarium*-infected PVX-free tubers revealed that approximately 47% of the isolated walls was cellulose (Fig. 3-A). Since potato cell walls from tissue free of *F. roseum* 'Avenaceum' contain only 26%

cellulose, it can be concluded that a sizeable portion of the noncellulosic polysaccharides had been removed from these cell walls during pathogenesis. About 29% of the cell walls from the *Fusarium*-infected PVX-free tubers could not be accounted for as cellulose or other polysaccharides. Cell walls from *Fusarium*-infected tubers infected with PVX appeared to contain only 21% cellulose. Also, 64% of these walls could not be accounted for as cellulose or other polysaccharides. These latter results may be attributed to deposition of lignin-like substances in the cell walls during pathogenesis and/or masking of the polysaccharides in these walls (8), rendering them resistant to hydrolysis and analysis by the procedures used.

It can be concluded that *F. roseum* 'Avenaceum' is capable of degrading the polysaccharides in potato cell walls during pathogenesis, but the resistance of PVX-infected tubers to dry rot does not appear to be related to the cell wall composition in the tubers prior to tissue invasion by *F. roseum* 'Avenaceum'.

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