# Sucrose and Cell Walls as Factors Affecting Phoma Storage Rot of Sugar Beet

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ABSTRACT

Sucrose concentration and resistance of sugar beet storage roots to *Phoma betae* increased with age. Growth of *P. betae* on isolated cell wall material in culture also increased with the age of roots, but the production of endopolygalacturonate *trans*-eliminase (endoPGTE) decreased up to 142 days, then increased. Cell walls from a fodder cultivar induced more endoPGTE than cell walls from two sugar cultivars. Commercial cultivars that were higher in sugar remained resistant, whereas the low sugar, fodder cultivar became more susceptible.

There was a significant negative correlation between the sucrose percentage and disease rating of defoliated and nondefoliated plants. *Phoma betae* produced more endoPGTE when cultured on cell wall material from defoliated than from nondefoliated plants. Enzyme production was not affected by sucrose percentage of root tissue or cultivar. Individual roots that expressed a resistant reaction to *P. betae* usually had a high sucrose percentage. But the association of resistant roots with resistance to maceration by culture filtrates and the production of endoPGTE on isolated cell wall material suggest that the properties of cell walls as well as sucrose content affect pathogenesis and the activity of endoPGTE.

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*Phoma betae* (Oud.) Frank, the imperfect stage of *Pleospora bjoerlingii* Byford, is an important storage rot pathogen of sugar beet (*Beta vulgaris* L.), causing a loss of sucrose from stockpiled roots (5, 8). Nelson & Oldemeyer (10) reported that roots with the lowest sucrose concentration had the highest amount of decay caused by *P. betae*. Recent data show that plants with the highest Cercospora leaf spot rating also had the greatest amount of decay in storage (14). This suggests that the sucrose concentration influences the rate of decay but does not establish the mode of action.

Reduced symptom severity resulted after the sugar concentration of some hosts was increased. This has been correlated with a decrease in the synthesis of cell-wall-degrading enzymes by the fungal pathogens (3, 9, 12). Increased in vitro synthesis of certain cell-wall-degrading enzymes also has been reported for fungi grown on walls from susceptible but not resistant host tissue (2, 7). The involvement of endopolygalacturonate *trans*-eliminase in Phoma storage rot has been demonstrated (4). The present study was conducted to determine the effects of sucrose concentration on the severity of Phoma storage rot in sugar beet, and the effects of sucrose and host cell walls on growth and enzyme production by the pathogen in vitro.

MATERIALS AND METHODS.—Three cultivars of *Beta vulgaris* L. 'American 2 hybrid B' (2B), 'American 3 hybrid N' (3N), and a fodder beet ('A58'), were planted in the field and harvested at various intervals. The first two cultivars are grown commercially in the Red River Valley of North Dakota and Minnesota. None of these cultivars has an acceptable level of resistance to *P. betae*. A58 is more susceptible than are the other two cultivars.

Sugar beet roots were inoculated with toothpick cultures of *P. betae*. Round toothpicks were boiled for 1 hr, dried, soaked 1 hr in potato-dextrose broth, placed in screw-top jars, and autoclaved. Each jar of toothpicks was inoculated with an agar plug 3 mm in diam taken from the edge of a colony of *P. betae*. The toothpick cultures were used for inoculation when fungal growth had covered the toothpicks (2-3 weeks). The area of the root to be inoculated was disinfested with 95% ethanol, inoculated, and incubated at 10 C and 92 to 98% relative humidity for 4 weeks. Each lesion was measured (in mm) along the longest and shortest axis, because the lesions often were oval-shaped with the longer axis being parallel to the vascular system. The sum of the two measurements were multiplied by five to give a disease rating. Six roots for each of four replicates for each harvest date were inoculated immediately after harvest. Sucrose determinations and cell wall material were prepared after harvest from a separate root from each replicate.

In one experiment, 2B and A58 were defoliated 9 weeks before harvest to simulate loss of leaf area from leaf spot pathogens. This results in sucrose depletion with the production of new leaves. A disease rating was determined for five roots from each of four replicates. The roots were then analyzed for sucrose using the lead acetate method (6). The most susceptible and resistant root from each cultivar that had or had not been defoliated was used to prepare cell wall material.

Cell walls used as a carbon source for *P. betae* were prepared according to the method of Nevins et al. (11) and used in a medium which contained: 4 g

cell wall; 2.4 g $(NH_4)_2SO_4$; 0.3 g $MgSO_4$; 1.5 mg $Fe(NO_3)_3$; 0.9 mg $ZnSO_4$; 0.4 mg $MnSO_4$; and 1,000 ml of distilled water. The pH was adjusted to 7.0 with 1 M KOH. Twenty ml were dispensed to each flask (containing 80 mg cell wall) and autoclaved. Thiamine (0.2 $\mu$g) was added through ultrafilters to each flask after autoclaving. This medium was found suitable for endopolygalacturonate *trans*-eliminase (endoPGTE) production (4).

Growth of the fungus in the cell wall medium was measured by determining the protein content of the mycelium because the suspended cell walls prevented an accurate dry weight of the mycelium. Incubation was at 25 C for 2 weeks. Each culture was filtered, and the mycelium was placed in 30 ml of cold 0.5 N NaCl and homogenized for 2 min in a high-speed tissue homogenizer. The mycelial suspension was cooled in an ice bath and further disintegrated by exposure to ultrasonic sound for 4 min. This was centrifuged at 3,500 *g* for 15 min, and the supernatant was measured for protein content in a double beam spectrophotometer using the method of Waddell (15).

Culture filtrates were centrifuged, then dialyzed in ca. 100 volumes of glass-distilled water at 4 C for 17 to 20 hr. Protein concentrations in the dialyzed culture filtrates were measured using the method of Waddell (15). The dialysate was tested for pectolytic enzyme activity by using a semimicro thiobarbituric acid (TBA) test (1), and tissue maceration ability.

The reaction mixture for the TBA test contained 5 ml of 1% pectin in 0.05 M Tris [tris (hydroxymethyl) amino methane] buffer at pH 8.5, 1 ml of 0.01 M $CaCl_2$ and 4 ml of dialyzed culture filtrate. After incubation at 30 C for 2 hr, the mixture was poured into 35-ml centrifuge tubes with 0.6 ml 9% $ZnSO_4$ and 0.6 ml 0.5 N NaOH. This was centrifuged for 15 min at 3,500 *g*. Three ml of 0.04 M TBA, 1.5 ml of 1 N HCl, and 0.5 ml of distilled water were added to 5 ml of the cleared supernatant. This was boiled in a bath for 30 min to develop the color. A spectral analysis showed that maximum absorption occurred at 550 nm, so measurements were made at this wavelength. The increase in absorbance per hr per ml of filtrate times 100 was divided by mg of protein per ml to give units/mg.

The maceration ability of the undialyzed culture filtrate was determined by measuring the loss of strength of tissue slices on a penetrometer as described by Sherwood (13). Root slices 0.25 mm thick and 14 mm in diam were placed in a reaction

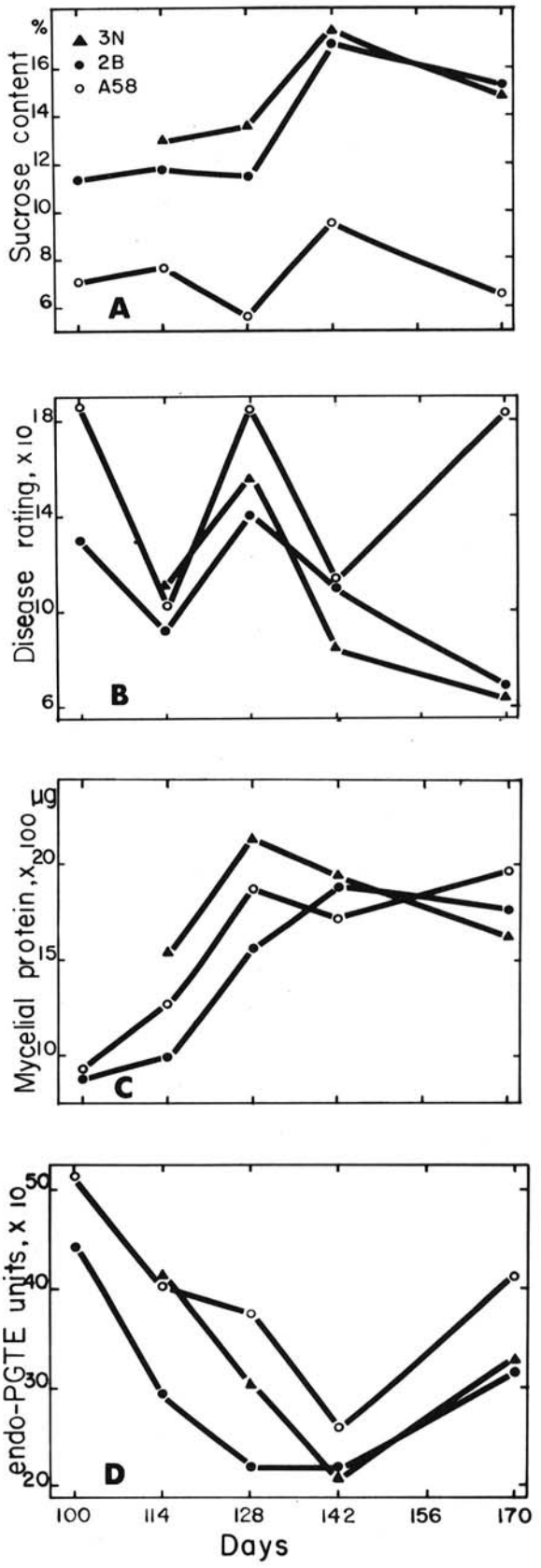


**Fig. 1.** The properties of roots of three cultivars of sugar beet during maturation from 100 to 170 days after planting in the field. **A)** Tissue sucrose as a percentage of fresh weight. **B)** Disease rating after inoculation with *Phoma betae*; 4 weeks' incubation at 10 C. **C)** Average growth of three cultures/cultivar from each harvest, measured as mycelial protein, of *P. betae* grown in liquid culture with isolated cell wall material as the sole carbon source. **D)** Specific units of endopolygalacturonate *trans*-eliminase (endoPGTE) produced by *P. betae* in culture with cell wall material as the carbon source.

mixture containing 10 ml of Tris buffer at pH 8.5 and 5 ml of undialyzed culture filtrate. This was incubated for 1.5 hr in a water bath at 30 C. The force required to burst the slices was recorded as grams of water and expressed as a percentage strength loss compared to slices placed in a reaction mixture with water instead of enzyme. Average strength loss was based on 10 slices/root.

RESULTS.—The disease ratings at the first harvest date were 131 to 188; A58 with the lowest sucrose had the highest disease rating. As the season progressed, there was a buildup of sucrose (Fig. 1-A). Exceptions were the temporary decrease at 128 days in A58 and the drop during the 142- to 170-day period. The first decrease cannot be explained, but the decrease during the final month was probably due to cloudy skies, a condition not favorable for sucrose storage. There was an increase in resistance of 2B and 3N to *Phoma* as the roots matured and sucrose increased. Resistance continued to increase during the last month, when there was a slight loss of sucrose. The disease rating of A58 fluctuated considerably during the course of the experiment. During the last month, there was an increase in susceptibility and a loss of sucrose (Fig. 1-A, B).

These results indicated inconsistent correlations between resistance to *Phoma* and sucrose content. An experiment was performed to see what effect cell walls might have on the activity of *Phoma*. These data showed that the growth rate of *Phoma* was greater on cell walls from older roots than from younger roots (Fig. 1-C). There was no statistical difference among cultivars at any one harvest date, except that cell walls of cultivar 3N at 128 days induced significantly more growth. This increased growth rate leveled off after the roots were over 128 days old.

The increased utilization of cell wall material was not accompanied by increased endoPGTE production; rather, the reverse was true. Up to 142 days after planting, cell walls induced less endoPGTE production by *P. betae* (Fig. 1-D). This may partly account for the increase in resistance of older roots. But during the last month, under low light intensity when the assimilation rate of sucrose decreased, the production of endoPGTE on cell wall material increased, even though the growth rate of the fungus remained constant. Cell walls from A58, the susceptible cultivar, induced more endoPGTE than the other two cultivars.

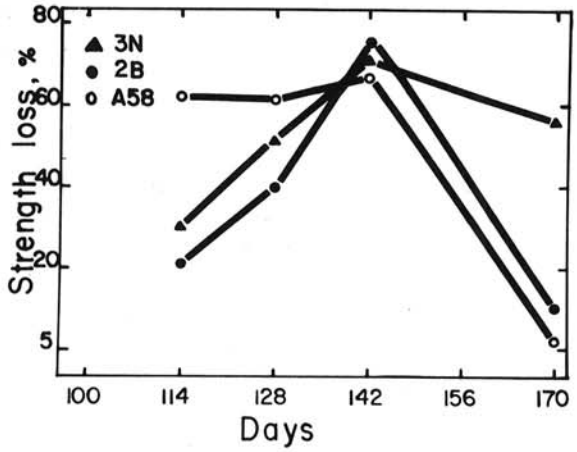


Fig. 2. Maceration of tissue slices expressed as percentage strength loss after exposure to undialyzed culture filtrates of *Phoma betae*.

TABLE 1. The effect of defoliation 9 weeks prior to harvest on sucrose content of sugar beet roots followed by infection with *Phoma betae*

| Cultivar | Defoliated | Sucrose (%) | Disease[a] rating |
|---|---|---|---|
| 2B | No | 16.2 a[b] | 124 b |
| | Yes | 14.8 ab | 140 ab |
| A58 | No | 10.2 bc | 145 ab |
| | Yes | 7.7 c | 154 a |

[a] Disease rating = the sum (in mm) of the longest and shortest distance of lesions at the root surface multiplied by five.

[b] Means followed by the same letter are not statistically significant at 5% (Duncan's new multiple range test).

As the roots matured up to 142 days, they became more susceptible to maceration, except for A58 which was considerably more susceptible than the other cultivars at 114 days of age (Fig. 2). The roots again became more resistant during the final 4 weeks. This response corresponds with the increase in resistance of whole roots of 2B and 3N to *P. betae*. The similar pattern of sucrose concentration and resistance to maceration indicates a possible relationship.

Defoliation lowered the sucrose content, but the decrease within each cultivar was not statistically significant (Table 1). The decrease in sucrose content was accompanied by lowered resistance, but again this was not significant. Evidently, 9 weeks was enough time for these plants to recover from defoliation and store sucrose in amounts comparable to the nondefoliated treatments.

The disease ratings and sucrose contents of defoliated (20 plants) and nondefoliated 2B (50 plants) were compared. The correlation coefficients showed a significant negative relationship between sucrose concentration and disease rating of defoliated ($r = .46$, 5% level of significance) and nondefoliated ($r = .41$, 1% level of significance) plants. Roots with the higher sucrose content had a lower disease rating regardless of defoliation.

One resistant and one susceptible root of cultivars 2B and A58 which were or were not defoliated were selected for further investigations into the sucrose-resistance relationship. Those plants that were most susceptible to *P. betae* also had the lowest sucrose content (Table 2). The small difference in percent sucrose between the resistant and the susceptible nondefoliated 2B (17.9 versus 17.0%)

TABLE 2. The effect of defoliation 9 weeks prior to harvest on the sucrose percentage, growth, and endopolygalacturonate *trans*-eliminase (endoPGTE) production by *Phoma betae* on cell wall material of sugar beets selected as resistant or susceptible to *P. betae*

| Cultivar | Defoliation | Disease rating[a] | Sucrose (%) | Mycelial protein (μg/culture) | endoPGTE (specific units) |
|---|---|---|---|---|---|
| 2B resistant | No | 45[a] | 17.9 | 1,372 bcd[b] | 206 |
| | No | 340 | 17.0 | 1,332 bcd | 162 |
| | Yes | 45 | 17.5 | 1,166 d | 250 |
| | Yes | 355 | 11.8 | 1,271 cd | 213 |
| A-58 susceptible | No | 60 | 11.0 | 1,271 cd | 217 |
| | No | 460 | 7.2 | 1,544 b | 210 |
| | Yes | 55 | 12.2 | 1,998 a | 251 |
| | Yes | 635 | 5.9 | 1,411 bc | 257 |

[a] Disease rating = the sum (in mm) of the longest and the shortest dimensions of lesions at the root surface multiplied by five.

[b] Means followed by the same letter are not significantly different at 5% level (Duncan's new multiple range test).

illustrate an exception to the general statement that susceptible roots are low in sucrose.

Cell wall material was prepared from these roots. The cell wall utilization by *Phoma* did not follow a pattern. The fungus grew equally well on all cell wall material of 2B regardless of defoliation or disease reaction. But *Phoma* was better able to utilize cell wall material from the defoliated, resistant root of A58 than from the defoliated susceptible root.

EndoPGTE production was affected by defoliation. A greater amount of endoPGTE was produced by *Phoma* when it was cultured on cell walls from defoliated plants (Table 2). This production had no relationship to the extent of mycelial growth, sucrose percentage, or cultivar.

DISCUSSION.—Data indicate that the sucrose concentration and properties of the host cell walls affect pathogenesis by *P. betae* in sugar beet storage roots. There was a significant negative correlation between disease ratings and sucrose concentrations. Isolated cell walls from roots 170 days old induced more endoPGTE production than roots that were harvested at 142 days, even though the growth rate of *Phoma* was comparable. English et al. (7) also found that the production of α-galactosidase and α-arabinosidase by *Colletotrichum lindemuthianum* was not proportional to the growth of the fungus.

The exact function of cell walls in regulating endoPGTE activity or production is not known. The ability of isolated cell walls to induce endoPGTE decreased as the roots increased in age to 142 days. From 142 to 170 days, this trend was reversed. Perhaps a cell wall property inhibitory to endoPGTE accumulated during root maturation and was removed or destroyed during the extraction process. This property also may have been altered by the production of new leaves, because cell walls from defoliated sugar beets induced more endoPGTE production in culture than nondefoliated plants.

A comparison of defoliated 2B with 11.8% sucrose and nondefoliated A58 with 11.0% sucrose shows that the growth rate and endoPGTE production by *Phoma* on cell wall material was comparable; yet, 2B in this particular case was much more susceptible than A58, even though the tissue sucrose was similar. Perhaps a mechanism of resistance was damaged by defoliation. If so, this mechanism is not obvious from the activity of *Phoma* on the isolated cell wall material.

The disease ratings and tissue maceration data imply that intact cells regulate enzyme activity and not necessarily production. The 170-day-old roots of cultivars 2B and 3N were more resistant than the younger roots, and tissue from roots of this age also were resistant to maceration by enzymes of culture filtrates. The exception to this is cultivar A58. Tissue of 170-day-old roots from this cultivar also was resistant to maceration even though the roots were susceptible to *Phoma*. The relatively low sucrose content or lack of other resistant mechanisms in vivo might account for this response.

During the final month before harvest, the sucrose concentration of all three cultivars decreased. However, the final sucrose concentration of cultivars 2B and 3N was still well within a commercially acceptable amount. During this period, resistance of the commercial cultivars 2B and 3N increased. If these roots were harvested at 142 days, they would have decayed faster than those harvested 1 month later. The resistance of tissue slices to maceration also increased during the last month. Apparently, as host tissue ages, it becomes more resistant to endoPGTE activity. A similar observation was made by Bateman et al. (2) with *Rhizoctonia solani* and bean tissue.

Some evidence herein shows that the sucrose content of sugar beet roots is associated with resistance to *P. betae*. Cell wall properties also influence the enzyme activity and growth of *P. betae*. Undoubtedly, there are other mechanisms involved. Certain individual roots of A58 and table beets low in sucrose express a resistant response to *P. betae* (W. M. Bugbee, *unpublished data*). Thus, selection of

roots for resistance to *P. betae* should only be based on the disease reaction and not on sucrose production.

## LITERATURE CITED


1. AYERS, W. A., G. C. PAPAVIZAS, & A. F. DIEM. 1966. Polygalacturonate *trans*-eliminase and polygalacturonase production by Rhizoctonia solani. Phytopathology 56:1006-1011.
2. BATEMAN, D. F., H. D. VAN ETTEN, P. D. ENGLISH, D. J. NEVINS, & P. ALBERSHEIM. 1969. Susceptibility to enzymatic degradation of cell walls from bean plants resistant and susceptible to Rhizoctonia solani Kühn. Plant Physiol. 44:641-648.
3. BIEHN, W. L., & A. E. DIMOND. 1970. Effect of carbon sources on the production of polygalacturonase by Ceratocystis ulmi. Phytopathology 60:1284 (Abstr.).
4. BUGBEE, W. M. 1972. Pectolytic enzyme production by Phoma betae. Can. J. Bot. 50:1705-1709.
5. CORMACK, M. W., & J. E. MOFFATT. 1961. Factors influencing storage decay of sugar beets by Phoma betae and other fungi. Phytopathology 51:3-5.
6. DE WHALLEY, H. C. S. (ed.) 1964. Cold digestion method, p. 79. Int. commission uniform methods sugar anal. Elsevier, Amsterdam. 153 p.
7. ENGLISH, PATRICIA D., J. B. JURALE, & P. ALBERSHEIM. 1970. Host-pathogen interactions. II. Parameters affecting polysaccharide-degrading enzyme secretion by Colletotrichum lindemuthianum grown in culture. Plant Physiol. 47:1-6.
8. GASKILL, J. O., & C. E. SELISKAR. 1952. Effect of temperature on rate of rotting of sugarbeet tissue by two storage pathogens. Amer. Soc. Sugar Beet Technol. Proc. 7:571-574.
9. KEEN, N. T., & J. C. HORTON. 1965. Sugar repression of endopolygalacturonase and cellulase synthesis during pathogenesis by Pyrenochaeta terrestris as a resistance mechanism in onion pink root. Phytopathology 55:1063-1064 (Abstr.).
10. NELSON, R. T., & R. K. OLDEMEYER. 1952. Preliminary studies applicable to selection for low respiration and resistance to storage rots of sugar beets. Amer. Soc. Sugar Beet Technol. Proc. 7:400-406.
11. NEVINS, D. J., P. D. ENGLISH, & P. ALBERSHEIM. 1967. The specific nature of plant cell wall polysaccharides. Plant Physiol. 42:900-906.
12. PATIL, S. S., & A. E. DIMOND. 1968. Repression of polygalacturonase synthesis in Fusarium oxysporum f. sp. lycopersici by sugars and its effect on symptom reduction in infected tomato plants. Phytopathology 58:676-682.
13. SHERWOOD, R. T. 1966. Pectin lyase and polygalacturonase production by Rhizoctonia solani and other fungi. Phytopathology 56:279-286.
14. SMITH, G. A., & E. G. RUPPEL. 1971. Cercospora leaf spot as a predisoposing factor in storage rot of sugar beet roots. Phytopathology 61:1485-1487.
15. WADDELL, W. J. 1956. A simple ultraviolet spectrophotometric method for the determination of protein. J. Lab. Clin. Med. 48:311-314.