Relationship Between Sorbitol and Solute Potential in Apple Shoots Relative to Fire Blight Symptom Development After Infection by *Erwinia amylovora*

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ABSTRACT

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The soluble carbohydrate (SC) content and solute potential (Ψ_s) of individual leaves on apple shoots were determined to test the hypothesis that these parameters might be related to the progress of fire blight symptom development after infection by *Erwinia amylovora*. Experiments were based on the use of leaves and shoots of the same physiological age from 1-yr-old Jonathan and Delicious apple trees, which are susceptible and normally resistant to fire blight, respectively. After inoculation of the individual leaves with a bacterial suspension containing 10^8 cfu/ml, symptom severity declined with increasing leaf age. Inoculations of the first three apical leaves of Jonathan shoots and the first two leaves of Delicious shoots all led to the invasion and subsequent death of the supporting shoot. No infections were established on shoots of either variety when inoculations were made below the fifth leaf. High-pressure liquid chromatography analyses of SC from individual leaves showed that this progressive expression of resistance with leaf age paralleled an increase in sorbitol concentration and an increasingly negative Ψ_s. In cultured apple shoots, tissue damage after tip inoculation progressed more rapidly in etiolated than in light-grown shoots. In both etiolated and light-grown cultured shoots, symptom severity declined with the accumulation of SC, particularly sorbitol, and increasingly negative Ψ_s when the carbon source in the medium, as sucrose or sorbitol, was increased. The Ψ_s of *E. amylovora* colonies in culture and of the bacterial ooze produced on inoculated immature pear slices is generally greater than the Ψ_s of young apple leaves but less than that of mature leaves. Thus, it appears that normal cell-maintenance functions in mature cells may confer resistance to damage by *E. amylovora* and that this mechanism is independent of the genetic resistance exhibited by the Delicious cultivar to fire blight in the field. This mechanism appears to explain why young tissues are more severely damaged by fire blight than mature tissues and provides a basis for understanding the renewal of infectious activity by the pathogen at canker margins in the spring.

Fire blight, caused by *Erwinia amylovora*, is one of the most destructive diseases of apples (*Malus* sp.) and pears (*Pyrus* sp.) worldwide. Steiner (25–27) characterized five distinct types of fire blight symptoms: blossom, shoot, canker, trauma, and rootstock blight. These types were based on sources of inoculum, conditions governing infection events, and the tissues involved. The extent to which tissue death proceeds from an infection site in each case is commonly seen to be greater in young tissues than in mature tissues. This report examines a physiological basis for symptom development in vegetative apple shoots infected by *E. amylovora* that appears to be related to the increasing solute potential (Ψ_s) that occurs with maturity.

Shoot blight is most destructive in young, nonbearing orchards where rapid vegetative growth is promoted to develop the basic tree structure. Significant damage can also occur in bearing orchards where infections initiated through young shoots often progress into and kill larger limbs and whole trees. An infected shoot wilts initially from the tip, resulting in the development of a characteristic crosier of the shoot tip. Droplets of a viscous ooze may be exuded onto the epidermal surfaces of the shoot and leaf petioles. The progress of the pathogen down the shoot axis occurs in advance of the discoloration and death of the attached leaves. In time, all or part of the shoot dies and develops a brown to blackish appearance.

One of the earliest symptoms of shoot infection at the cellular level is plasmolysis, which results in the displacement of air by water in the intercellular spaces around parenchyma cells in the young shoot cortex (1). In 1913, Bachmann (1) speculated that osmotic forces, in response to the bacteria and their ooze (extracellular polysaccharides and cell sap) in the intercellular spaces, were responsible for this damage. Nixon (19) confirmed Bachmann's observations and noted that as infections progressed into more mature tissues, less plasmolysis occurred and the bacterial cells in the intercellular spaces "appeared smaller and stained more deeply" than those in tissues undergoing damage. No clear explanation was given for this latter observation.

When Burrill (6) originally named the fire blight pathogen *Micrococcus amylovorus,* its association with low starch in infected tissues was the basis for the species epithet, *amylovorus.* While Hewitt (11) found a positive correlation between the starch content of host tissues and fire blight susceptibility in apples and pears, Stewart (28) demonstrated the inability of *E. amylovora* to utilize starch. Blake (5) suggested that succulent vegetative shoots, whose growth is induced by high rates of nitrogen, have low carbohydrate concentrations and, as such, are more susceptible to *E. amylovora.* Conversely, shoots with dark green leaves that have ceased growing have higher carbohydrate levels and are less susceptible. The focus of all these early experiments was on the osmotically inactive starch moiety, and no assays were attempted for osmotically active soluble carbohydrates (SC).

Since *E. amylovora* lacks the enzyme systems needed to degrade host cell walls (23), the mechanism of pathogenesis remains unclear. Bachmann's (1) observations on host cell plasmolysis, therefore, warrant more detailed study. For this, an examination of the role of osmotically active compounds in host tissues relative to the bacteria in the intercellular spaces is critical.

Of the many SC in apple tissues, the six-carbon polyol, sorbitol (D-glucitol), constitutes up to 80% of the photosynthate produced in apples and other Rosaceae plants (4). Sorbitol is found in all apple tissues and has been variously characterized as a storage product (30), an intermediate metabolite (14), a respiratory substrate (10), a cryoprotectant (4,20), and an osmoregulator (4). Sorbitol is preferentially loaded into the phloem of mature leaves and exhibits a strong source-sink mobilization within apple trees (3) from reserve tissues to growing shoot tips and fruits. In addition, sorbitol is reported to be required by *E. amylovora* for ooze production (2), which in turn is generally acknowledged as a pathogenicity factor (2,8,21). Finally, Johnson and Lakso (12) noted that for 22–25 days after bud break, young shoots are net importers of stored carbohydrates, primarily as sorbitol, and that as the shoots become net exporters, 20% of their energy demands continues to be supplied from reserves. The dynamics and timing of this flux in SC within apple tissues has not been examined with respect to fire blight symptom development. The objectives of this study were to determine 1) the relationships between the susceptibility to *E. amylovora* of vegetative apple tissues at various levels of maturity with respect to the amounts of SC, especially sorbitol, and the Ψ_s at the time of infection and 2) how these factors might influence the progress of symptom development.

MATERIALS AND METHODS

Bacterial culture. Strain Ea 581 of *E. amylovora* (obtained from Tom van der Zwet, U.S. Department of Agriculture, Agricultural Research Service, Appalachian Fruit Research Station, Kearneysville, WV) was used throughout this study. Cultures were grown routinely on nutrient yeast-dextrose agar (NYDA) medium at 26 C and transferred to fresh media 24 h prior to use in all experiments.

Planting material. One-year-old Jonathan and Delicious apple trees on seedling roots (Stark Brothers' Nurseries and Orchards, Louisiana, MO) were used for all greenhouse studies. Trees were grown in 4.6-L plastic pots with a commercial potting material, Progrow 300S (Progrow Products, Elizabeth City, NC), watered on a daily basis, and fertilized once a month with 10-10-10 (N-P-K) fertilizer. Studies were conducted on actively growing shoots having either eight or 12 leaves per shoot.

Selection of apple shoots. The plastochron index, as described by Erickson and Michelini (9), was used to establish equivalent physiological ages for the shoots and leaves of both cultivars. According to the index, Jonathan and Delicious shoots were determined to be equivalent at 1.9 days for the emergence of each new leaf. On this basis, actively growing shoots with the same number of leaves were accepted as being of equivalent phylogenic and physiological age for these experiments. Similarly, leaves from the same position on different shoots having the same number of total leaves were also considered to be physiologically equivalent.

Carbohydrate analysis of leaves. Leaves from Jonathan and Delicious apple trees were harvested at the eight- or 12-leaf stage, transported to the laboratory over dry ice, and then stored at −70 C until they were analyzed. Each sample consisted of three leaves from each leaf position, and each sampling was replicated three times. For each sample, cell sap was extracted with a common kitchen garlic press. The crude sap extract was centrifuged (model TJ-6, Beckman Instruments, Columbia, MD) at 1,500 *g* for 15 min to remove cell and tissue fragments. A 0.1-ml aliquot of the supernatant was diluted in 0.9 ml of distilled, deionized water and centrifuged again at 1,500 *g* for 10 min. The resulting supernatant was forced through a 0.22-mm nylon filter by syringe prior to analysis by high-pressure liquid chromatography (HPLC). Throughout this extraction procedure, the cell sap fraction was kept on ice at or near 0 C. Preliminary trials comparing the above extraction procedure with one in which boiling 80% ethanol (v/v) was used for 30 min to extract the cell sap resulted in equivalent amounts of SC. We elected to use the simpler and faster water-extraction protocol, since the potential for enzymatic destruction of various SC was apparently not significant.

The clarified samples were analyzed for SC with a high-pressure liquid chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with LC-6A pumps, a 6A RID refractive index detector, and an SIL-6B autosampler. Separation of the SC was accomplished with a 300- × 7.8-mm HPX-85C carbohydrate column (Bio-Rad Chemical Division, Richmond, CA) at 85 C with helium-sparged distilled, deionized water at 0.6 ml/min as the mobile phase. The carbohydrates were quantified by using a refractive index detector to compare the refractive index of each sample with an external standard. All data were stored and analyzed on a Shimadzu C-R4A Chromatopac integrator. Concentrations of SC were converted to Ψ_s equivalents by using the van't Hoff relationship:

$$\Psi_s = (-nRT)/V,$$

where n is the number of solute molecules in solution of volume, V; T is absolute temperature; and R is the gas constant.

Determination of Ψ_s in apple leaves and bacterial ooze. Leaves were harvested from eight- or 12-leaf shoots and stored at −70 C. The leaf sap was then extracted with a garlic press. The Ψ_s of a 0.10-ml aliquot of the undiluted, crude sap from each leaf was determined immediately without centrifugation with a model 5500 vapor pressure osmometer (Wescor Instruments, Logan, UT). To minimize water loss, this procedure was conducted in a cold room at 4 C with 100% relative humidity. The Ψ_s of *E. amylovora* cultures grown on NYDA medium amended with 4 g of asparagine and 1 g of sorbitol per liter (2) was determined by transferring actively growing cultures from the agar surface to the osmometer with a glass rod. This amended NYDA medium was chosen because the ooze produced on it is reported to be spectrophotometrically similar to that obtained from infected apple tissues (2). Similarly, the Ψ_s of bacterial ooze produced on immature Bartlett pear slices inoculated with *E. amylovora* was collected with a micropipette and transferred immediately to the osmometer. For this latter test, cross-sectional slices of immature, surface-sterilized pears were placed in 100- × 80-mm petri dishes and inoculated with a bacterial suspension containing 10^8 cfu/ml. The slices were maintained at 26 C and 100% relative humidity in the closed dishes for 72 h for ooze production. As with the leaf sap, all measurements of the ooze Ψ_s were made at 4 C and 100% relative humidity.

Leaf susceptibility to *E. amylovora*. Leaves of the same age from eight- or 12-leaf shoots were selected from trees in the greenhouse and inoculated while on the tree with a suspension of *E. amylovora* (approximately 10^8 cfu/ml) in a phosphate buffer at pH 6.5. Inoculations were done by cutting 3 mm from the leaf tips and dipping these cut ends into the bacterial suspension for 3 s. Similarly cut controls were dipped in buffer only. Three replicates of each leaf age on eight- and 12-leaf shoots were inoculated.

The inoculated plants were kept in the greenhouse and evaluated at 5 and 7 days after inoculation to determine the number of leaves infected and the leaf necrosis index (LNI) of each leaf. The LNI was used to quantify symptom severity, where LNI = (length of necrotic tissue on leaf axis/total length of leaf axis) × 100. The leaves were also classified as susceptible (LNI > 60), moderately susceptible (LNI = 15–60), and resistant (LNI < 15).

Expression of shoot susceptibility in vitro. Axillary buds from 5- to 7-cm shoots of Jonathan and Delicious apples were excised, washed for 24 h in half-strength Murashige and Skoog (MS) liquid medium (16), and placed on half-strength semisolid MS medium for 3 days at 25 C. The buds were then transferred to covered glass dishes containing solid MS medium amended with sucrose or sorbitol at either 30 or 60 g/L to precondition them to a specific carbon source. After 3 wk, the apical tips of shoots were removed aseptically and transferred to fresh medium for shoot proliferation at 25 C with a 16-h photoperiod under white fluorescent lights with a photon flux density of 40–60 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This apical tip subculturing procedure was repeated two more times at 4-wk intervals. At the last subculture, half of the culture

dishes were shielded from light with aluminum foil to prevent photosynthesis and to reduce the supply of stored photosynthates. The other half of the cultures were exposed to light as indicated. After 4 wk, 20 2-cm shoot axes (shoots without leaves) of each cultivar were selected from each treatment for analyses of SC by HPLC separation. This analysis was repeated twice. Tissues were homogenized in distilled, deionized water in a 15-ml glass tissue grinder (Corning Glass Works, Corning, NY), and the extract was adjusted to a total of 10 ml. The suspension was centrifuged (Beckman model J-21B) at 23,000 *g* for 20 min. The resulting pellet was twice resuspended in 5 ml of distilled, deionized water and centrifuged again as above. The combined supernatants from all three extractions were placed in a single scintillation vial and evaporated to dryness with a Speedvac model svc100 vacuum dryer (Savant Instruments, Farmington, NY). The dried residue was then resuspended in 1.0 ml of distilled, deionized water and centrifuged for 10 min. The resulting supernatant was filtered through a 0.22-μm nylon filter before HPLC.

Inoculation of cultured shoots was done by excising 2 mm of tissue from each shoot tip and placing a droplet of bacterial suspension (10^8 cfu/ml) on the cut end with a hypodermic needle. Five and 7 days after inoculation, the number of shoots infected and the severity of necrosis (shoot necrosis index [SNI]) were determined. The SNI was calculated as (length of necrotic shoot tissue/total length of shoot) $\times$ 100.

Statistical analysis. The data on vegetative shoots were analyzed by general linear models and regression. Equality of regression forms was determined by an F^* test (18). For treatments where the regression lines were not significantly different at $P \leq 0.05$, the data were pooled for analysis. Analysis of variance as a four-factor, completely randomized design was used to analyze data from tissue-culture studies.

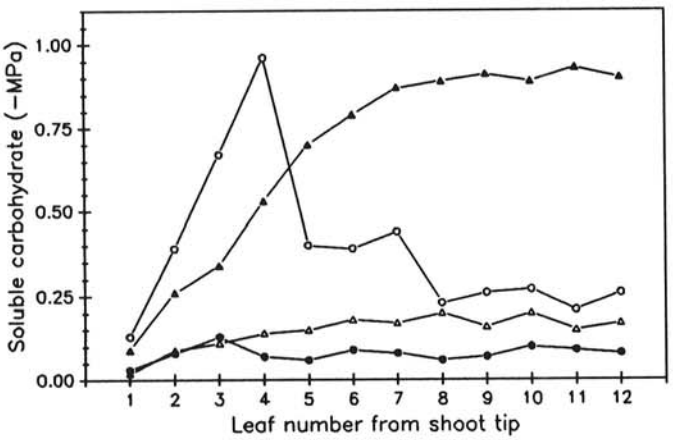

Fig. 1. The concentration of soluble carbohydrates expressed as MPa of solute potential in leaves of Jonathan apple shoots with 12 leaves. Leaf 1 is the youngest leaf. The values represent the means of three replicates at each leaf position. ○ = Glucose; ● = sucrose; △ = fructose; and ▲ = sorbitol.

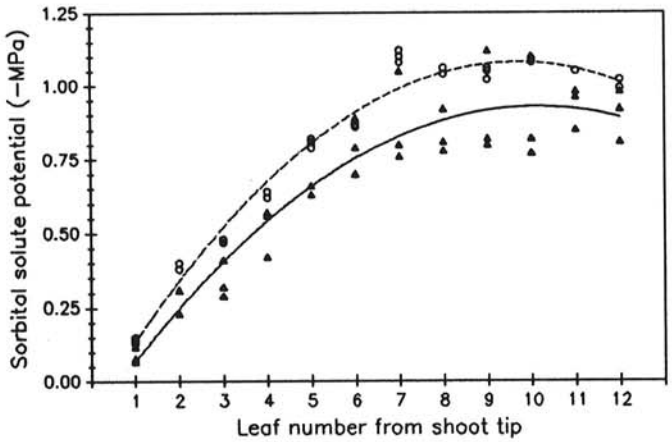

Fig. 2. The relationship between sorbitol concentration, expressed as units solute potential (MPa), and leaf number for Jonathan (▲) and Delicious (○) apple shoots with 12 leaves.

RESULTS

Carbohydrate analysis of leaves. Analyses of apple leaves for SC composition showed the presence of sucrose, glucose, fructose, sorbitol, and trace amounts of raffinose and stachyose for both cultivars. Glucose was the predominant sugar in leaves 1–4 from Jonathan shoot tips (Fig. 1), and similar results were obtained in Delicious shoots (data not shown). Below the fourth leaf on each shoot, sorbitol was the most abundant SC for both cultivars. The regression equations for the relationship between leaf number and sorbitol for 12-leaf shoots are

$$y = -0.096 + 0.210x - 0.012x^2$$

for Jonathan ($R^2 = 0.90$) and

$$y = -0.130 + 0.240x - 0.010x^2$$

for Delicious ($R^2 = 0.98$), where x represents the leaf number and y is the sorbitol concentration converted to MPa to express Ψ_s (Fig. 2). The two regression forms between leaf number and sorbitol are not significantly different at $P = 0.05$ for both cultivars. Table 1 shows statistical values for the pooled data for the two cultivars relative to leaf age and the SC measured. The relationships between leaf age and sucrose, fructose, and total SC were not significant at $P = 0.05$. However, the relationships between leaf age and sorbitol and glucose were significant at 0.01 and 0.05, respectively.

Solute potential of apple leaves and bacterial ooze. Although the data in Table 2 show that the leaf-to-leaf Ψ_s values for Delicious leaves 1–6 (−1.21 to −1.58 MPa) were slightly higher than those for Jonathan shoots (−1.01 to −1.47 MPa), this difference was not significant at $P = 0.05$. The regression equations for Ψ_s in the leaves are

$$y = -0.89 + 0.012x - 0.005x^2$$

for Jonathan ($R^2 = 0.90$) and

$$y = -1.14 + 0.082x - 0.003x^2$$

for Delicious ($R^2 = 0.94$) (Fig. 3). Analyses of the pooled data on Ψ_s for both cultivars showed a highly significant R^2 value (Table 1), and the regression form is represented by the equation

$$y = -0.98 + 0.118x - 0.006x^2.$$

The Ψ_s of the bacterial ooze produced on inoculated slices of immature pears averaged -1.51 ± 0.01 MPa, while that of

TABLE 1. Coefficient of determination and significance[a] of polynomial regressions of carbohydrate composition against leaf necrosis and leaf age of the pooled data for the cultivars Jonathan and Delicious apple trees in vivo

	Leaf necrosis index		Leaf age	
	R^2	P	R^2	P
Carbohydrate				
Sucrose	0.56	*	0.19	NS
Glucose	0.02	NS	0.50	*
Fructose	0.77	**	0.05	NS
Sorbitol	0.98	***	0.81	***
Total soluble carbohydrate	0.51	*	0.36	NS
Total solute potential (Ψ_s)	0.88	***	0.90	***
Leaf number	0.96	***		

[a] *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$; and NS, not significant.

TABLE 2. Comparison of leaf necrosis index (LNI), sorbitol concentration, sorbitol, and total soluble carbohydrate (SC) solute potential (Ψ_s), and the ratios of the Ψ_s of sorbitol to total SC and host cell to bacterial ooze for Jonathan and Delicious apple leaves of the same physiological age

	Jonathan						Delicious					
Leaf no.[a]	LNI	Sorbitol (mg/ml)	Ψ_s (−MPa) Sorbitol	Ψ_s (−MPa) Total SC	Sorbitol-total SC Ψ_s ratio[b]	Host-ooze Ψ_s ratio[c]	LNI	Sorbitol (mg/ml)	Ψ_s (−MPa) Sorbitol	Ψ_s (−MPa) Total SC	Sorbitol-total SC Ψ_s ratio	Host-ooze Ψ_s ratio
1	66.3	6.5	0.09	1.01	0.089	0.68	65.0	10.7	0.14	1.21	0.116	0.82
2	68.1	19.1	0.26	1.12	0.232	0.76	61.0	28.4	0.39	1.28	0.116	0.86
3	64.0	26.3	0.34	1.25	0.272	0.85	54.0	34.2	0.47	1.36	0.346	0.92
4	41.2	39.1	0.53	1.31	0.405	0.89	43.0	46.5	0.63	1.45	0.434	0.98
5	29.0	51.1	0.70	1.43	0.490	0.97	12.0	59.1	0.81	1.58	0.513	1.07
6	9.2	58.2	0.79	1.47	0.537	0.99	0.8	63.8	0.87	1.58	0.551	1.07
7	3.1	64.7	0.87	1.57	0.554	1.06	0.0	81.0	1.10	1.54	0.714	1.04
8	1.8	60.8	0.81	1.58	0.513	1.07	0.0	77.2	1.05	1.55	0.677	1.05
9	0.9	67.1	0.90	1.57	0.573	1.06	0.0	76.3	1.04	1.64	0.633	1.11
10	1.0	65.7	0.88	1.56	0.564	1.05	0.0	79.9	1.08	1.66	0.651	1.12
11	1.0	66.2	0.89	1.67	0.533	1.13	0.0	77.1	1.05	1.72	0.610	1.16
12	1.0	62.9	0.85	1.70	0.500	1.15	0.0	74.3	1.01	1.70	0.594	1.15

[a] Leaf position from shoot tip.
[b] Proportion of the Ψ_s represented by total SC that is contributed by the sorbitol moiety.
[c] Based on a measured Ψ_s of −1.48 MPa for *Erwinia amylovora* cultures on nutrient yeast-dextrose agar medium amended with asparagine and sorbitol.

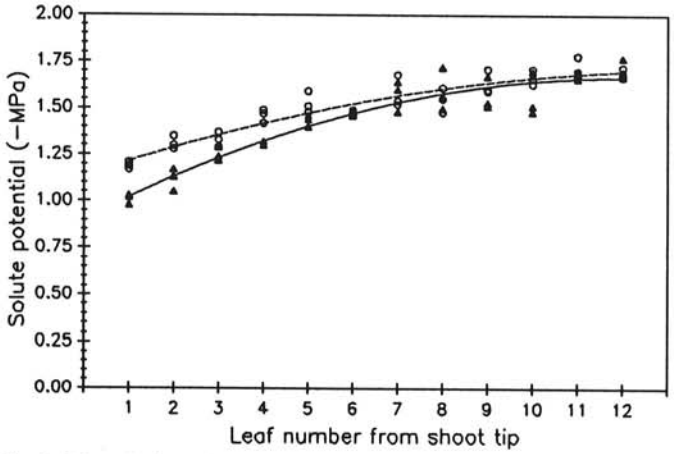

Fig. 3. The relationship between measured solute potential (MPa) and leaf position for Jonathan (▲) and Delicious (○) apple shoots with 12 leaves.

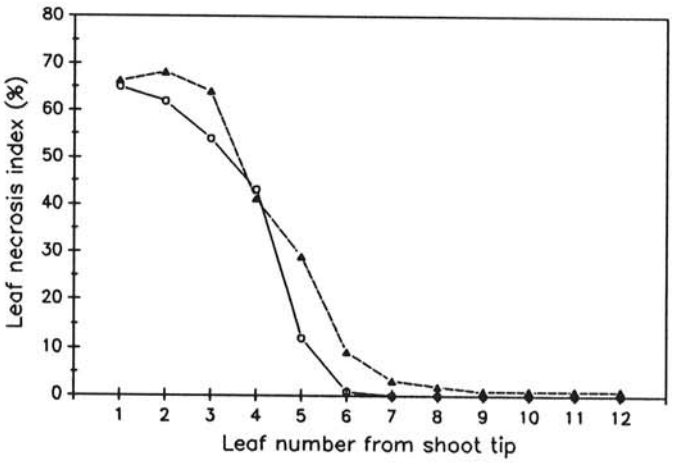

Fig. 4. The relative susceptibility of Jonathan (▲) and Delicious (○) apple leaves of various ages to *Erwinia amylovora*. Leaf age is designated as leaf number beginning from the shoot tip. The leaf necrosis index was determined for each leaf at 7 days after inoculation for 12-leaf shoots. Each value represents the mean of three replicates.

cultures on more chemically defined medium averaged −1.48 ± 0.01 MPa.

Susceptibility of leaves to *E. amylovora*. Tissue discoloration and necrosis were used to determine whether infection with *E. amylovora* had occurred. As discoloration of tissue progressed into the petiole, leaves either flagged or rolled abaxially; wilting and necrosis of the entire leaf followed. Infections initiated on leaves 1–3 on Jonathan shoots and on leaves 1 and 2 on Delicious shoots resulted in the complete blighting of the entire shoot, while those initiated on the fourth Jonathan and third Delicious leaf from the shoot tip were limited to the inoculation wound. Leaves at the sixth Jonathan and fifth Delicious nodes and below showed strong resistance.

There was a significant decrease in LNI with increasing leaf maturity in each cultivar on 12-leaf shoots (Fig. 4). Similar results were observed with eight-leaf shoots of both cultivars (data not shown). The regression forms for LNI and leaf age were not significantly different at $P = 0.05$ between cultivars and shoot age. The models for LNI and each of the SC concentrations except glucose (pooled data) were significant at $P \leq 0.05$ (Table 1). Sorbitol had the best fit relationship on the basis of R^2 values between LNI and each of the SC.

Susceptibility of tissue-cultured shoots. Shoots of both cultivars were successfully grown in tissue-culture medium containing 3% sucrose and 3% sorbitol. At 6% sucrose or 6% sorbitol, Jonathan shoots were slightly more vigorous (growth rate and shoot length) than those of Delicious. Analyses of the shoot axis tissues for SC indicated the presence of sorbitol, glucose, sucrose, fructose, and trace amounts of stachyose. The SC concentrations, regardless of shoot type (etiolated or light-grown), increased with increased levels of the carbon source in the medium. The concentrations of sorbitol, glucose, fructose, and sucrose were significantly different between 1) the Jonathan and Delicious cultivars ($P \leq 0.05$), 2) the etiolated and light-grown shoots of each cultivar ($P = 0.01$), and 3) the carbon sources ($P = 0.01$). The concentrations of sorbitol, glucose, and fructose were significantly different between carbon source levels at $P = 0.01$ (Table 3). The differences between cultivar and shoot type interactions were not significant for sorbitol.

Shoots of both cultivars inoculated under culture conditions were susceptible to *E. amylovora*. There was no significant difference ($P = 0.05$) between the number of etiolated and light-grown shoots of either cultivar that developed infections after inoculation (data not shown). The symptoms expressed were similar to those observed in the greenhouse on susceptible cultivars, but those on etiolated shoots developed within 48 h while those on light-grown shoots developed within 72 h after the inoculation. As the carbohydrate concentration in the medium increased, the sorbitol concentration in shoots increased while the SNI declined

(Table 4). The disease severity (as SNI) on etiolated shoots, however, was significantly greater than that on light-grown shoots of each cultivar in that symptoms developed more slowly on the latter (Table 4). There was also no significant difference ($P = 0.05$) between the light-grown shoots of the two cultivars with respect to disease severity.

DISCUSSION

The cytology of fire blight symptoms in apple tissues after infection by *E. amylovora* was reviewed recently by Schouten (22). Despite the detail available, however, there has been no unified physiological model that explains why the progress of symptoms ceases in some tissues without the formation of a physical barrier and then begins again at some later time. In 1913, Bachmann (1) observed that the first symptom of infection in young apple shoots inoculated with *E. amylovora* was the plasmolysis of cortical parenchyma cells. That event led to the subsequent loss of turgor, cell collapse, and death of cells in the cortex and eventually to the invasion of xylem elements. She speculated that this initial effect was caused by the withdrawal of water from the cells via osmosis as influenced by the presence of bacterial ooze in the surrounding intercellular spaces. Nixon (19) later concurred with Bachmann's report and added that as infections proceeded into more mature tissues, the amount of plasmolysis decreased, and the bacteria in the intercellular spaces appeared smaller and stained more deeply.

The results of this study confirm Bachmann's early hypothesis by defining the relationship of Ψ_s to symptom development. These data provide the basis for a physiological model that explains changes in the susceptibility of different tissues to damage by *E. amylovora* at different times during the season and presents evidence that sorbitol is the principal SC governing this mechanism. It is suggested that Nixon's (19) observations on the appearance of the bacteria in the intercellular spaces in more mature tissues might well be the result of reduced ooze formation at these sites.

Sorbitol is the predominant end product of photosynthesis in mature apple leaves (4). It is synthesized from glucose-6-phosphate by the enzyme NADP-sorbitol-6-phosphate dehydrogenase (15). However, since young, photosynthetically immature leaves lack this enzyme, they import sorbitol as an energy source from mature leaves and reserve tissues and convert it to fructose via the enzyme sorbitol dehydrogenase (17), which is found largely in immature leaves and fruits, creating strong metabolic sinks to which sorbitol is rapidly mobilized from older tissues (17). Figure 1 shows the steady increase in sorbitol with leaf maturity; a stable level is reached at about the seventh leaf from the tip. The abrupt rise in glucose in the first four young leaves at the shoot tip is most probably the result of its accumulation in the absence of the enzyme needed to produce sorbitol for export. These findings are all consistent with those reported elsewhere (3,30).

Table 2 shows an increase in the Ψ_s of the leaf cell sap that is concomitant with the increase in sorbitol concentration and leaf maturity. The high R^2 values for the regression analyses between leaf age, LNI, and Ψ_s suggest that leaf susceptibility decreases as the pattern of sorbitol production increases and overall Ψ_s becomes more negative. The close relationship between the Ψ_s and symptom severity (LNI) indicates that host cell damage in response to *E. amylovora* may be dependent on differences in the Ψ_s between cell sap and the bacteria with their ooze in the intercellular spaces. The Ψ_s of the bacterial ooze produced by *E. amylovora* on pear slices and on NYDA-asparagine-sorbitol media (2), which is reported to be spectrophotometrically similar to ooze from infected apple tissues, averaged −1.51 and −1.48 MPa, respectively. In Table 2, the latter value is used to calculate ratios of leaf Ψ_s to ooze Ψ_s. Where these ratios were <0.86 and >1.0, the leaves were very susceptible (LNI > 60% with sorbitol concentrations of <30 mg/ml) and very resistant (LNI < 15% with sorbitol concentrations of >55 mg/ml), respectively. While the measurements of total Ψ_s of cell sap represent all osmotically active components, sorbitol appears to be the predominant factor governing this mechanism in both cultivars such that when the ratio of sorbitol to total SC Ψ_s is >0.5, resistance is expressed (Table 2).

TABLE 3. *F* values[a] from analysis of variance for soluble carbohydrates of etiolated and light-grown shoots of Jonathan and Delicious apple cultivars cultured in vitro on Murashige and Skoog medium amended with sorbitol and sucrose at 3 and 6%

Source of variation[b]	df	Soluble carbohydrates			
		Sorbitol	Glucose	Fructose	Sucrose
C	1	326.9**	20.9**	6.7*	275.5**
ST	1	994.9**	29.6**	35.9**	47.9**
C × ST	1	NS	73.6**	20.5**	39.0**
CS	1	763.9**	112.9**	204.9**	14.3**
CS × ST	1	6.4*	7.3*	30.9**	36.1**
CS × C	1	147.6**	NS	NS	33.7*
CS × ST × C	1	37.7**	NS	NS	31.4**
CL	1	500.5**	31.3**	31.3**	NS
CL × ST	1	182.3**	NS	9.6**	5.0*
CL × C	1	45.7**	NS	NS	NS
CL × CS	1	16.9**	7.2*	NS	NS
C × CS × CL	1	NS	9.9**	NS	5.1*
CS × ST × CL	1	17.3**	NS	5.5*	5.1*
C × CL × ST	1	NS	4.3*	10.9**	NS
C × CS × CL × ST	1	17.6**	6.6**	7.3**	NS
Error	32				
CV		11.4	18.9	18.3	32.0

[a] *, $P = 0.05$; **, $P = 0.01$; and NS, not significant at 0.05.
[b] C = cultivar; ST = shoot type; CS = carbon source; and CL = carbon level.

TABLE 4. Effect of carbon source and light on soluble carbohydrate composition and necrosis in cultured stem tissues of Jonathan and Delicious apple shoots infected by *Erwinia amylovora*

Carbon source[a]			Jonathan					Delicious				
				Soluble carbohydrates[d] (mg/g, fresh weight)					Soluble carbohydrates (mg/g, fresh weight)			
Sucrose (%)	Sorbitol (%)	Shoot type[b]	SNI[c]	Sorbitol	Glucose	Fructose	Sucrose	SNI	Sorbitol	Glucose	Fructose	Sucrose
3	0	L	65.3	6.1	8.5	8.1	7.4	55.0	6.2	11.6	5.8	1.0
		E	90.1	0.5	9.6	10.2	0.2	85.0	0.9	8.4	8.0	1.4
6	0	L	35.0	17.7	8.6	6.0	12.4	45.2	28.3	20.2	9.2	2.0
		E	85.4	0.9	13.6	15.4	0.7	90.4	1.3	4.8	11.7	0.8
0	3	L	65.0	15.3	4.7	3.5	9.2	35.1	25.3	11.6	4.8	0.4
		E	95.2	3.2	3.9	4.4	8.9	85.1	14.2	2.4	2.6	0.0
0	6	L	25.0	30.5	6.5	3.8	9.1	35.2	43.1	10.5	5.6	0.8
		E	75.3	4.7	7.2	6.1	8.3	80.0	28.8	4.0	4.0	1.0

[a] Carbon source was either sucrose or sorbitol at 3 or 6% (w/v).
[b] L = light-grown; and E = etiolated, dark-grown.
[c] Shoot necrosis index as percentage of shoot length showing symptoms.
[d] Measured by high-performance liquid chromatography. Values are averages of three replicate samples.

On this basis, we conclude that when the Ψ_s of the pathogen and its polysaccharide matrix in the intercellular spaces is more negative than that of the surrounding host cells, the osmoticum allows water to be extracted so that the cells collapse and die, resulting in the appearance of visible symptoms. Conversely, when the Ψ_s of the pathogen and its ooze in the intercellular spaces is equal to or less than the tissue Ψ_s, host cells retain their turgor, water and nutrients are withheld from the bacteria, and symptoms do not develop. This osmotic model for symptom development is consistent with the views of Schouten (21) and Shaw (24), who both held that the availability of water to the pathogen in the intercellular spaces was a requirement for pathogenesis.

Further evidence that the host cell osmoticum limits the damage caused by *E. amylovora* where colonization occurs well in advance of symptoms is the fact that this natural defense mechanism may be breached by simple wounding. Steiner (*unpublished data;* 29) noted that, despite the use of aseptic techniques, cutting out infected limbs in advance of visible symptoms leads to the rapid development of new bark cankers around the wounds. Such localized wounding may release the osmotic control of water movement between host and pathogen, making water and cell sap freely available for colonization by the bacteria. Furthermore, as these newly established bark cankers progress into intact, mature tissues, cell damage again becomes limited.

Once infections were established at the tips of either cultured or greenhouse-grown shoots, their downward progress was similar in both the fire blight-susceptible Jonathan and the normally resistant Delicious cultivars (29). This indicates that resistance to damage by *E. amylovora* at the cellular level is probably conferred by the normal maintenance functions of mature cells. The leaves on shoots of both cultivars demonstrated moderate resistance to leaf infection in the greenhouse at the fourth to fifth leaf position, and leaves at the sixth position and lower showed a high level of resistance. These results are consistent with those of Crosse et al (7) for Jonathan leaves. That no significant difference was shown in the susceptibility of the two cultivars except at the third leaf position is attributed to the high levels of inoculum (10^8cfu/ml) used throughout these experiments to ensure the initial establishment of infections. In this regard, Crosse et al (7) noted a direct relationship between inoculum concentration and infection efficiency in Jonathan leaves. Additional research needs to be pursued to determine whether the high level of resistance exhibited in the field with Delicious shoots also might be related to the amount of primary inoculum introduced at the infection site.

The above physiological model for symptom initiation, development, and cessation is further supported by this study, in which apple shoots were grown in vitro. Symptoms in both cultivars developed more rapidly in etiolated shoots, where photosynthesis was prevented and sorbitol levels were lower than in light-grown shoots. The differences in the sorbitol concentrations accumulated in etiolated and light-grown shoots were significant and are attributed to qualitative and quantitative differences in the sources of carbon available rather than to the absence of photosynthesis. Of particular interest is the fact that when sorbitol was the sole carbon source, etiolated Delicious shoots had four to six times more sorbitol than similarly etiolated Jonathan shoots. This suggests that either the usually resistant Delicious shoots are more efficient than Jonathan shoots in the production and mobilization of sorbitol or that Jonathan shoots are able to convert sorbitol to other carbohydrates more readily. Such differences alone, however, do not explain the strong resistance of Delicious to infection in the field.

This study was limited to the progress of infections and symptom development in current season shoots. Nevertheless, given the mobility of sorbitol throughout the apple tree system (3,4,30), it is reasonable to expect that the same osmotic mechanism described here might also operate at the margins of overwintering cankers. On the basis of a reliably predictable interval of 90 degree-days $\geq$12.7 C between infection and fire blight symptom appearance (25,26), Steiner (27) used regression analysis to estimate the renewal of infectious activity by *E. amylovora* at the margins of overwintering cankers at approximately 40 degree-days $>$12.7 C after the green tip stage of apple bud development. This timing is quite consistent with the mobilization of sorbitol from reserves in bark tissue to the peak demand for carbohydrates by young apple shoots at 22–25 days after bud break reported by Johnson and Lakso (12). Further research is needed to determine whether, as suggested in this study, the mobilization of sorbitol away from overwintering bark canker margins results in a host Ψ_s greater than -1.0 MPa, which would allow the bacteria at these sites to acquire the water necessary for pathogenesis.

The osmotic model proposed here is intended to describe pathogenesis at the cellular level only. It does not preclude and, indeed, is compatible with other theories based on the activity of specific gene products at the molecular level in the pathogen (31) and in the host (13). Further investigation of the possible osmotic effects of sorbitol on the bacterial cells is required using plant systems in which sorbitol concentrations in the host tissues might be varied. This could be done, for example, by measuring the relative susceptibility of vegetative shoots on trees with various amounts of fruit (i.e., additional sorbitol sinks) or by partially girdling portions of limbs to reduce their supply of photosynthates. Yet another approach would be to inoculate single shoots on supporting limbs of different sizes and ages and to measure the extent to which subsequent infections progress into the larger limbs as a function of SC reserves present.

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