Carbohydrate Changes in Tobacco Systemically Protected Against Blue Mold by Stem Infection with *Peronospora tabacina*

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ABSTRACT

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Soluble carbohydrates increased in tobacco leaves and stems that were protected against blue mold by stem inoculation with sporangia of *Peronospora tabacina*. Increases ranged from 1.1-fold to eightfold by 3 wk after inoculation. Most of the increase was in free glucose; lesser increases in fructose and a variable increase in sucrose were found. Disruption of phloem translocation of photosynthate or ontogenic changes caused by the stem infection did not account for the changes in sugar levels. Starch content was not reduced, and invertase or amylase activities were not elevated, except in necrotic stem tissues where starch decreased and amylase and invertase activities increased. These changes in necrotic stem tissues were insufficient to account for the sugar increases found throughout immunized plants. Crude extracts from infected tissues of inoculated stems liberated significantly greater amounts of soluble carbohydrate from tobacco stem cell wall preparations than did extracts of uninfected tissues. Administration of exogenous sugars to tobacco plants by various means did not consistently affect susceptibility of tobacco to blue mold. Neither glucose nor fructose inhibited germination of *P. tabacina* sporangia at physiological concentrations, but fructose inhibited at levels approximately sixfold the highest levels detected in tissue.

It has long been recognized that levels of soluble carbohydrates in plants have a pronounced influence on microbial infection and that infection may alter the carbohydrate metabolism of infected plants (2,8,11,16,39). Horsfall and Dimond (16) introduced the concept of "high-sugar" and "low-sugar" diseases; that is, certain plant diseases are favored by high or low concentrations of soluble carbohydrates. Numerous reports have described plant diseases apparently favored by high (5,10,14,27,36) or by low (7,17,26,29,45,46) concentrations of soluble sugars. Lyles et al (27) found that highly resistant and highly susceptible near-isogenic cultivars of wheat apparently differ phenotypically only in soluble carbohydrate levels, and Daly (7) reported that the administration of exogenous soluble sugars reduces or enhances resistance.

However, many factors have complicated considerations of roles for soluble carbohydrate in plant disease resistance. Investigators have reported inconclusive or even contradictory results with the same or similar host-pathogen combinations (13,14,26,45) or varying results based on cultivar, age, or plant tissue (5,18,19). Most investigations have described only plant sugar levels before pathogen inoculation or at various, often unspecified, infection stages. Some have failed to report the infection or disease status of plant tissues analyzed. Sugar levels may differentially influence spore germination, penetration of the host, development of the pathogen within the host, and, ultimately, reproduction and dissemination of the pathogen (21,31). After infection, microbes, especially biotrophic pathogens, may in turn markedly alter source-sink relationships, respiration, catabolic and anabolic enzymes, or photosynthetic activity, thus creating a nonadditive joint host-pathogen carbohydrate metabolism (2,7,18,19,22). Levels of pathogen pressure or disease severity may differentially influence changes in carbohydrates (21). Furthermore, in vivo soluble carbohydrate levels are inextricably connected to both external and internal factors including past and present light intensity and quality, day length, water relations, nitrogen and other inorganic nutrition, temperature, hormonal levels, tissue type and location, age, and ontogenic processes such as flowering. All of these also can influence or be influenced by microbial-plant interactions.

Thus, although it is evident that soluble carbohydrates may have a role in influencing the course of plant diseases, the subject is exceedingly complex and controversial.

We report here a pronounced systemic increase in soluble carbohydrates in infected and uninfected tissues of tobacco plants "immunized" against blue mold by a limited stem infection with *Peronospora tabacina* (4,6,42). We report our findings on the nature of the elevated soluble carbohydrates and their possible sources and influence upon susceptibility of tobacco to blue mold. Preliminary reports have described aspects of this study (32,33).

MATERIALS AND METHODS

Plants and pathogen. Tobacco plants (*Nicotiana tabacum* L. 'Kentucky 14 White Burley') and the pathogen (*Peronospora tabacina* Adam, isolate Spindletop 82) were maintained, and tobacco plants were stem-inoculated, challenged, and rated for disease symptoms as previously described (34).

Girdling. Girdling of stem tissue to block phloem transport frequently increases sugars in tissues above the girdle. Because stem inoculation with *P. tabacina* causes restricted necrosis in the stem, the effect of girdling on soluble carbohydrate was considered. Surgical girdling of plants was done by excising with a razor blade a band of stem tissue external to the woody xylem approximately 5–8 cm in width and approximately 10 cm above the soil line. In total girdling, the entire circumference of the plant was cut; in partial girdling, two patches totaling about three-fourths of the circumference were removed on opposite sides of the stem. Tissues removed included the epidermis, cortex, outer phloem, and cambium. Remaining tissues were the pith, including the inner phloem, and the xylem. Plants were mechanically weakened by this operation and required staking for support, but they did not wilt and otherwise continued to grow and develop normally.

Administration of exogenous sugars to plants. Aqueous solutions of glucose, fructose, or sucrose were prepared in distilled deionized water and sterilized by filtration through a 0.22-μm-

pore-diameter membrane filter (Millipore Corp., Bedford, MA). In one set of experiments, 1.5 ml containing 75 mg/ml of glucose, 10 mg/ml of fructose, or 15 mg/ml of sucrose was injected with a hypodermic syringe (No. 2 needle) three times weekly into the petioles and stems of young vegetative plants (25–30 cm initial height) for 3 wk. Plants injected with water or sporangia of *P. tabacina* served as controls. Leaf and stem tissues were collected for determinations of sugar content, and plants were challenged and rated for disease. In another set of experiments, one petiole per plant of young (4–5 true leaves) tobacco plants was severed under water and a vial containing 10 ml of sugar solution (5–10% w/v of glucose, sucrose, or fructose) was fastened over the petiole stump on the plant. The vial was replaced daily for a week, and the end of the stump was trimmed daily to permit continued uptake of sugar solution by the plant. The leaves and stems were then analyzed for sugar content, challenged, and rated for disease. In a third series of experiments, young (4–6 true leaves) plants were sprayed on either dorsal (adaxial) or ventral (abaxial) leaf surfaces with sugar solutions (5% w/v of glucose, fructose, or sucrose; approximately 5 ml/leaf, 25 ml/plant). The solutions were allowed to dry, and the plants were challenged on the dorsal surface with the pathogen and rated for disease.

In vitro spore germination. Sporangia were collected with a damp sable artist's brush from sporulated lesions on small (2–4 leaf stage) plants and then suspended in cold distilled deionized water. The spore suspension was filtered through cheesecloth, and the spores were washed with sterile water and resuspended several times on an 8.0-μm-pore-size membrane filter (Millipore Corp.). The washed spores were resuspended in water, and the concentration was adjusted to 2×10^4 sporangia/ml with the aid of a hemacytometer. Ten microliters of the suspension (approximately 200 sporangia) was then pipetted onto the surface of an agar disk (1.8% w/v Phytagar [GIBCO Laboratories, Grand Island, NY], 1.2-cm diameter, about 0.42-ml volume). The agar gel contained added sugars (see Results) or no additives (water agar) as a control. Inoculated agar disks were incubated in the dark for 24 hr at 18 C, 100% relative humidity. Germination of sporangia was determined using 100× magnification of a light microscope.

Tissue sampling and analysis for sugars. Leaf tissues were sampled by periodically excising small areas of the lamina of young but mature leaves (leaf position number 3 to 5 from the apex) with scissors, avoiding large veins. Stems were sampled by excising with a razor blade patches of tissue external to the woody xylem including the epidermic, cortex, outer phloem, and cambium. Samples were extracted immediately or frozen at −20 C. No significant differences in soluble sugar content or composition were found between fresh and frozen tissues.

Fresh or unthawed frozen tissue samples were weighed rapidly and then immersed in boiling 80% v/v aqueous ethanol and boiled for about 10 min. After cooling, the volume was adjusted for evaporation losses, and the tissue-ethanol slurry was homogenized with sand in a mortar and pestle. The homogenate was filtered through Whatman GF/A glass fiber filters (Whatman Ltd., Maidstone, England), and total soluble carbohydrate was estimated spectrophotometrically by the phenol-sulfuric acid method (9). Glucose in 80% v/v aqueous ethanol was used as a standard, and results are expressed as glucose equivalents. Individual saccharides were detected and semiquantitatively estimated by descending paper chromatography (solvent: *n*-butanol-pyridine-water, 6:4:3 v/v) with detection by alkaline silver nitrate (40). Quantitative determinations of glucose, fructose, mannose, and sucrose were made by the coupled glucose-6-phosphate dehydrogenase enzymatic assay (12). Starch was extracted with perchloric acid, precipitated as the iodine complex (1), and estimated in water solution as glucose equivalents by the phenol-sulfuric acid method.

Extraction of plant tissues for crude enzyme preparations. Freshly excised leaf or stem tissues (10 g) were homogenized with sand and a mortar and pestle at 0–4 C in a 25-ml buffer. The buffer (pH 6.8) contained 0.1 M potassium phosphate, 2% w/v bovine serum albumin (Sigma Chemical Co., St.Louis, MO), 0.2% w/v 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate detergent (Sigma Chemical Co.) and 2 g of suspended acid-washed polyvinylpolypyrrolidone (PVPP) (Sigma Chemical Co.). The resulting slurries were filtered through cheesecloth and Whatman GF/A filter paper, centrifuged at 20,000 *g* at 4 C for 20 min, and dialyzed at 4 C for 48 hr against three changes of 20 mM, pH 6.5, potassium phosphate buffer containing suspended PVPP. Dialyzed solutions were then recentrifuged and used as crude enzyme preparations.

Enzyme activity determinations. Amylase (starch hydrolase) and invertase (sucrase, sucrose hydrolase) activities were determined by measuring release of reducing sugars from 10 mg/ml of "soluble" potato starch (Sigma Chemical Co.) or 50 mM sucrose, respectively, in 20 mM, pH 6.5, potassium phosphate buffer at 22 ± 2 C. Reducing sugars were estimated as glucose equivalents by alkaline copper arsenomolybdate (37). Cellulase activity was estimated by solubilization at pH 6.5 of Cellulose Azure (Sigma Chemical Co.) (30). Alpha-glucosidase, β-glucosidase, and β-*N*-acetylglucosaminidase activities were estimated, after alkalinization of the reaction mixture with 0.2 NNaOH, by spectrophotometric determination at 410 nm of *p*-nitrophenolate released from the respective *p*-nitrophenylglycosides (8 mM) at 22 ± 2 C in 20 mM pH 6.2, potassium phosphate. All reaction mixtures contained 25 μl of crude enzyme preparation/ml. Enzyme units were calculated as μmol product released per minute (amylase, invertase) or ΔOD/min (cellulase, other glycosidases).

Digestion of cell wall preparations. Water-washed whole stem sections were homogenized in a Waring blendor at 0 C in 10 ml of 0.5N NaCl/g fresh weight. The resultant slurry was centrifuged at 5,000 *g* at 4 C. The pellet was resuspended, rehomogenized, and recentrifuged twice in 0.5 N NaCl and three times in ice water. The pellet was then suspended 30 min in boiling 95% v/v aqueous ethanol. The ethanolic slurry was filtered and the collected solids were resuspended and washed twice with hot 95% v/v aqueous ethanol. The solid residue was heated 30 min in chloroform-methanol 2:1 v/v to remove residual lipids, filtered, and rinsed with acetone and diethyl ether. The resultant fibrous, off-white material (stems) was air dried and used as a cell wall preparation. For the assays, 500 mg of stem cell wall preparation was incubated with 2 ml of crude enzyme preparation from stem tissue in 50 mM phosphate buffer (pH 6.2) at room temperature.

RESULTS

Soluble carbohydrate increase. Limited infection of tobacco stems with *P. tabacina* significantly elevated levels of soluble carbohydrates in stem and leaf tissues as compared with levels in uninfected (control) plants (Table 1). Necrotic stem tissues of stem-infected plants were higher in soluble carbohydrates than stem tissues of control plants but significantly lower than green uninfected stem tissues of stem-infected plants. Soluble carbohydrate in tissues of stem-infected plants ranged from 110 to 800% of control plants (not shown) with mean values of 260% for

TABLE 1. Effects of stem infection with *Peronospora tabacina* upon levels of total soluble carbohydrates in tobacco tissues[a]

Tissues	Soluble carbohydrates, as glucose equivalent[b] (μmol/g dry wt.)
Leaves	
Controls	230
Stem-infected	598*
Stems	
Green tissues	
Controls	160
Stem-infected	530**
Necrotic tissue	
Stem-infected	258*

[a] Data represent means of 14 independent experiments performed throughout 2 yr, 6 to 16 plants per treatment per experiment.

[b] *, significantly different from respective controls at $P = 0.05$, by Student's *t* test, **, $P = 0.01$.

TABLE 2. Effects of stem infection with *Peronospora tabacina* on the content of the major monosaccharides and disaccharides in tobacco tissues[a]

Tissue	Glucose[b] (μmol g/dry wt.)	Fructose (μmol g/dry wt.)	Sucrose (μmol g/dry wt.)	Recovery[c] (%)
Leaves				
Controls	109	52	26	84
Stem-infected	197*	97**	29	87
Stems				
Green tissues				
Controls	66	37	34	98
Stem-infected	266**	69**	50*	108
Necrotic tissues				
Stem-infected	86	35	15	66*

[a] Data represent means of eight independent experiments performed throughout the year, 6 to 12 plants per treatment per experiment.
[b] *, Significantly different from respective controls at $P = 0.05$, by Student's t test; **, $P = 0.01$. Analysis done on normalized data to compensate for large absolute differences among experiments.
[c] Amount of the total soluble carbohydrates, estimated by the phenol-sulfuric acid method as glucose equivalents, which is accounted for by the enzymic assay glucose, fructose, and sucrose. Balance may represent oligosaccharides.

TABLE 3. Effects of surgical girdling and stem infection with *Peronospora tabacina* upon soluble sugar contents in tobacco tissues[a]

Tissue	Soluble carbohydrate[b] (μmol glucose equivalent g/dry wt.)
Leaves	
Controls	315
Stem-infected	750*
Fully girdled	370
¾ girdled	385
Stems	
Controls (not girdled, not infected)	56
Stem-infected	
Green (above necrosis)	242*
Necrotic area	180*
Fully girdled (above girdle)	102*
¾ girdled (above girdle)	49

[a] Data represent means of triplicate determinations upon pooled tissue samples from 6 plants in each treatment.
[b] *, Significantly different from respective controls at $P = 0.05$, Student's t test.

TABLE 4. Effect of stem infection with *Peronospora tabacina* on the starch content of tobacco tissues[a]

Tissues	Starch (μmol glucose equivalent g/dry wt.)
Leaves	
Controls	102 ± 59
Stem-infected	136 ± 52
Stems	
Green tissues	
Controls	24 ± 9
Stem-infected	21 ± 8
Necrotic tissues	
Stem-infected	17 ± 10

[a] Data represent means ± standard deviations of determinations from seven independent experiments. Each experiment included 6 plants per treatment. Differences between stem-infected plants and their appropriate controls are not statistically significant.

leaves and 330% for stems. The magnitude of increase appeared to correspond with light intensity in the greenhouse. Lowest values were obtained during heavily overcast weather of winter and early spring, and highest values were obtained during bright, sunny days of late summer and autumn. However, in 14 independent experiments each with six to 14 plants per treatment over the course of almost 2 yr, soluble carbohydrate was consistently and significantly higher in stem-infected plants than in control plants.

Most of the increase in soluble carbohydrate was in glucose with a lesser increase in fructose (Table 2). Increases in sucrose were modest and inconsistent in leaves and uninfected stem tissues of stem-infected plants. Sucrose levels were reduced in necrotic infected stem tissues. Glucose plus fructose plus sucrose accounted for approximately 85% of the total soluble carbohydrate (as determined by phenol-sulfuric acid) in leaves and for virtually all of that in non-necrotic stem tissues (Table 2). In necrotic stem tissues of immunized plants, however, an average of only about two-thirds of the total soluble carbohydrate was accounted for by the three sugars. Paper chromatography (data not shown) revealed a number of ill-resolved oligosaccharides but no other monosaccharides or disaccharides in any tissue. Oligosaccharides may account for the balance of the phenol-sulfuric acid-detectable carbohydrates.

Possible sources of elevated soluble carbohydrate. Surgical girdling of tobacco stems resulted in increased soluble carbohydrates in leaf and stem tissues above the zone of girdling (Table 3). However, the increase was considerably less than that observed for stem-infected plants.

Under our experimental conditions, analyses of leaf and stem tissues collected from stem-infected or control plants at different stages of maturity through the period of flowering indicated that the difference in carbohydrate in stem-infected plants was not due to enhanced maturation (data not shown). Differences in soluble carbohydrate levels between stem-infected and control plants persisted through flowering.

Analyses of both stem-infected and control plants revealed insignificant differences in starch contents in immunized plants (Table 4). The amount of starch found in control plants was insufficient to provide the increase in glucose observed in stem-infected plants.

Examination of amylase (starch hydrolase) and invertase activity levels revealed decreased levels in leaf and green stem tissues of stem-infected plants relative to control plants (Table 5). In necrotic stem tissues, increased invertase activity (Tables 5 and 6) correlated with reduced sucrose contents (Table 2), but increased amylase activity (Tables 5 and 6) did not correspond with significant reduction in starch (Table 4). Other saccharide hydrolytic enzyme activities increased in necrotic stem tissues (Table 6).

These findings did not eliminate the enzymatic hydrolysis of polysaccharides in infected stem lesions as a possible source of elevated soluble carbohydrates in stem-infected plants. Extracts of cortical tissues from stem lesions released significant amounts of soluble carbohydrate from tobacco stem cell walls, but preparations from green tissues of control stems did not (Table 7). The nature of the solubilized carbohydrates is under investigation. Polygalacturonase activity was detected in the extracts of cortical tissues with stem lesions.

Effects of exogenous sugars on resistance of plants to blue mold. Administration of exogenous glucose, fructose, sucrose, or mixtures to whole tobacco plants through uptake by severed petioles did not cause any significant increase in resistance to blue mold (Table 8). Increases in soluble carbohydrates in plant tissues resulting from exogenous sugar administration ranged from 60 to

TABLE 5. Effect of stem infection with *Peronospora tabacina* upon activities of amylase and invertase in tobacco tissues[a]

Tissue	Amylase[b,c] (units g/fr. wt. tissue)	Invertase[bc] (units g/fr. wt. tissue)
Trial 1		
Leaves		
Controls	0.14 ± 0.01	0.11 ± 0.01
Stem-infected	0.07 ± 0.01*	0.10 ± 0
Stems		
Controls	0.11 ± 0.03	0.05 ± 0
Stem-infected		
Necrotic	0.25 ± 0.05*	0.14 ± 0.01*
Trial 2		
Leaves		
Controls	0.35 ± 0.04	0.31 ± 0.02
Stem-infected	0.14 ± 0.03*	0.20 ± 0.04*
Stems		
Controls	0.22 ± 0.02	0.05 ± 0.01
Stem-infected		
Green	0.10 ± 0.02*	0.04 ± 0.01
Necrotic	0.36 ± 0.03*	0.08 ± 0.01*

[a] See Materials and Methods for definitions of enzyme units and details of enzyme extraction and assay.
[b] Values represent means ± standard deviations of triplicate determinations on 3 samples.
[c] *, Significantly different from respective controls at $P = 0.05$, Student's t test.

TABLE 6. Glycohydrolase activities in necrotic infected stem tissues and stem tissues of uninfected plants[a]

Enzyme activities	Uninfected (units/g fr. wt.)	Infected (necrotic)[b] (units/g fr. wt.)
Amylase	0.16 ± 0.03	0.30 ± 0.06*
Invertase	0.05 ± 0.01	0.11 ± 0.02*
α-glucosidase	5.6 ± 2.2	7.6 ± 3.0
β-glucosidase	9.3 ± 0.7	10.3 ± 0.1
β-N-acetyl-glucosaminidase	34 ± 7	78 ± 8*
Cellulase	not detected	not detected

[a] See Materials and Methods for details of assays. Values represent means ± standard deviations of triplicate determinations upon pooled tissue samples from 6 plants per treatment.
[b] *, Significantly different from value for uninfected plant tissues at $P = 0.05$, Student's t test.

150 μmol glucose equivalent/g dry weight. The highest increases attainable through exogenous sugar administration were significantly less than the average increase in stem-infected plants (370 μmol/g dry weight) but still well within the range of values found in immunized plants (20–1,750 μmol glucose equivalent increase g/dry weight). Similar results were seen when solutions were injected into petioles or stems (data not shown).

Spraying 5% w/v solutions of glucose, fructose, or sucrose on upper or lower surfaces of leaves before inoculation had inconsistent effects on blue mold susceptibility (data not shown). In one experiment, blue mold infection was significantly decreased; in two others, it was little changed or slightly increased; in a fourth, it was markedly increased. Phenol-sulfuric acid assays of aqueous alcoholic washings of intact leaves from stem-infected and control tobacco plants revealed minor differences in soluble carbohydrates and generally very low concentrations (stem-infected: 9.0 ± 0.3; controls: 11.9 ± 2.3 μmol glucose equivalent g/dry weight). However, homogenization and extraction of leaves after surface washing with aqueous alcohol did not affect differences between stem-infected and control plants in soluble carbohydrate levels (immunized: 511 ± 39; controls: 189 ± 27 μmol glucose/g dry weight).

In vitro germination of *P. tabacina* sporangia upon agar disks was virtually unaffected by 0.1 or 1% w/v glucose, fructose, or sucrose or 5% w/v glucose or sucrose (63 to 80% germination; water controls, 81%). Five percent fructose was significantly

TABLE 7. Digestion of tobacco stem cell walls by crude enzyme preparations from stem tissues infected with *Peronospora tabacina* and from stems of uninfected plants[a]

Cell walls incubated with:	μmol glucose equivalent solubilized/g dry wt. cell walls[b]
Buffer alone	14 ± 0
Preparation from controls	16 ± 1
Preparation from infected plants	44 ± 4

[a] Enzyme preparations are dialyzed buffer-detergent extracts of stem tissues, prepared as described by Materials and Methods. Data reported were obtained after incubation of enzyme and cell wall preparations for 48 hr.
[b] Values represent means ± standard deviation of triplicate assays from two experiments.

TABLE 8. Effects of feeding sugars through petioles on the susceptibility of tobacco to blue mold[a]

Treatment	Total soluble carbohydrate in leaves after feeding (μmol glucose equivalent/g dry wt)	Disease	
		Percent leaf area symptoms	Spores/cm² of leaf
Water	157	57	23,300
5% glucose	234	58	15,100
10% glucose	218	55	23,400
5% fructose	267	42	38,900
10% fructose	285	52	24,000
10% sucrose	261	49	15,500

[a] Data are means of triplicate determinations upon 6 plants. Differences in disease were not statistically significant.

inhibitory (32% germination). However, this concentration (277 mM) greatly exceeds that to which germinating *P. tabacina* sporangia would be exposed in vivo, either on leaf surfaces or within leaf tissues. The maximum in vivo fructose concentration determined in either control or stem-infected plant tissue was approximately 45 mM (or 0.8% w/v).

DISCUSSION

Relatively little is known about the effects of carbohydrate levels on infection by downy mildews or about effects of infection upon carbohydrate metabolism in plants (20,35). In many rust and powdery mildew infections, a pronounced increase in soluble carbohydrate and starch in zones of fungal growth has been reported from penetration of the host to sporulation (2,21,44). However, this local increase has generally been accompanied by a decrease in starch and soluble carbohydrate in uninfected plant tissues and, frequently, also by a systemic increase in invertase and amylase activities postulated to function in mobilizing plant carbohydrates for transport to the fungal parasitic "sink" (3,7,15,24,28,38,44). These observations contrast with our findings of pronounced systemic increases in soluble carbohydrates and negligible changes in starch in tobacco plants stem-infected with the downy mildew *P. tabacina*. If the restricted stem lesion generated a "sink" for photosynthate, it certainly did not deplete the remainder of the plant. In further contrast with the rusts and powdery mildews, we measured decreases in invertase and amylase activities in tissues other than the lesion itself (Table 5). Although glucose and fructose increased in stem-infected plants (Table 2), glucose increase was disproportionate to the fructose increase, suggesting that sucrose hydrolysis was not the source of the increase in reducing sugars. The increase in glucose in stem-infected plants was often greater than the total soluble carbohydrate content present in corresponding control plant tissues.

These data suggested a net systemic increase in total soluble carbohydrate in stem and leaf tissues of stem-infected plants caused by the "infection" and not simply a redistribution from uninfected to infected tissues. Possible causes of this increase that

we considered included: 1) occlusion of the phloem by the stem lesions, causing photosynthate to be trapped within the shoot rather than being transported to the roots; 2) ontogenic changes in carbohydrate metabolism resulting from the accelerated morphogenesis frequently produced by blue mold stem infection (4,6,34,42); and 3) mobilization of sugars from sucrose or starch or other polysaccharides, e.g., cell walls, either systemically or from the lesion.

Surgical girdling intended to mimic or even exceed the degree of possible blockage or destruction of the outer phloem by fungal stem lesions did produce a modest increase in soluble carbohydrates in tissues above the girdle (Table 3). However, the increase was substantially less than that produced in stem-infected plants. Thus, occlusion of the phloem by stem infection is unlikely to be responsible for more than a modest fraction, 10–15% at most, of the elevated soluble carbohydrate in stems and leaves of stem-infected plants.

It has been reported that tobacco plants gradually increase in soluble carbohydrate concentrations as they age (23,41). Stem-infected tobacco plants generally grow faster, flower earlier, and senesce more rapidly than controls (34,42). However, the magnitude of increased soluble carbohydrate, particularly in stem tissues, reported for aging Burley tobacco (41) is less than that of the difference we found between control and stem-infected tobacco plants. Under our conditions, we found no significant increase in carbohydrate in our greenhouse-grown plants related to aging or onset of flowering (data not shown). Furthermore, increased carbohydrate levels for stem-infected plants persisted through flowering and onset of senescence even when physiological age was comparable between stem-infected and control plants (data not shown). Amylase and invertase activities have been reported to increase in tobacco plants with age (41). This finding contrasts with our observations of decreases in these activities in uninfected tissues of stem-infected tobacco plants (Table 5). These observations suggest that accelerated physiological aging of stem-infected tobacco plants is not a significant factor in causing elevated accumulation of soluble carbohydrates.

The negligible difference in starch found in tissues of stem-infected tobacco plants makes it unlikely that net mobilization of glucose from starch reserves is a source of elevated soluble sugars. Although extracts of infected stem tissues were effective in liberating soluble carbohydrate from tobacco stem walls (Table 7), quantitative calculations showed that total conversion of all starch, sucrose, and cell wall polymers in the volume occupied by typical stem lesions could not produce the quantities of soluble carbohydrates necessary to systemically elevate sugars to the concentrations found in stem-infected plants. Furthermore, analyses of necrotic tissues revealed no significant reduction in starch and a loss of only about two-thirds sucrose (Table 2). Although cortical and xylem tissues atrophy in advanced stem lesions, leading to stem brittleness, the destruction of the stem tissues is far from complete.

Dark fixation of CO_2 via the malic enzyme, similar to Uromyces rust of beans (7,47) was not directly investigated but is unlikely due to the large net amounts of sugar accumulated systemically. Although rust fungi may contribute to localized starch granule accumulation by heterotrophic CO_2 fixation, they must do so at the expense of metabolites translocated from other plant tissues, thus resulting in a net systemic loss.

The net increase (up to 40%) in dry weight of field-grown, stem-infected tobacco plants compared with uninfected controls (42,43) would seem prime facie evidence for significantly enhanced net photosynthesis by stem-infected plants. Conceivably, if, under "normal" conditions, sinks consume most of the photosynthate as rapidly as it is produced, the transit pool of soluble carbohydrates may be relatively small. A modest increase in photosynthesis, unaccompanied by a proportional increase in sink consumption, could result in a large proportional increase in the transit pool. Alternatively, stem-infected plants may have lower respiration rates in the dark. However, it is puzzling why the increase should be in glucose and fructose, not sucrose.

At this time, we cannot definitively state the precise source or sources of the striking systemic increase in soluble carbohydrates in tobacco plants stem-infected with *P. tabacina*. Partial occlusion of the phloem may contribute a portion; enzymatic degradation of polysaccharides in the stem lesion may contribute a further modest amount. The most reasonable source of the bulk of the increase is elevated net photosynthesis. We are investigating the effect of stem infection with *P. tabacina* on the photosynthesis of tobacco plants.

If indeed the concept of "high-sugar" and "low-sugar" diseases applies to blue mold of tobacco, it is unclear in which category the disease should be placed. High light intensity and physiological aging of tobacco decrease susceptibility to blue mold (25; Salt, Reuveni, Kuć, and Siegel, *unpublished*) suggesting that it may be a low-sugar disease. On the other hand, high nitrogen fertilization promotes susceptibility, a common characteristic of high-sugar diseases. Lucas (25) has shown that high soluble carbohydrate content in infected leaves clearly is necessary for efficient sporulation by the fungus, but this is distinct from susceptibility to infection. Administration of glucose, fructose, and sucrose to whole tobacco plants did not have a significant effect on susceptibility to blue mold (Table 8). The increases of soluble carbohydrates produced in plant tissues by exogenous administration were, however, lower than the average produced by stem infection, though still within the observed range. Applications of sugars to plants by spraying of leaves had very inconsistent results. Effects of surface applications of sugars may have been complicated by partial uptake into plants, degradation by, or effects on, epiphytic microbes, and direct effects on germinating *P. tabacina* sporangia.

In summary, it seems unlikely that moderately elevated levels of glucose, fructose, or sucrose play a significant direct role in induced resistance of tobacco to blue mold. It is yet of interest to definitively determine the in vivo source(s) of the elevated sugars, whether very elevated levels may play some role in resistance, direct or indirect, and whether they may be a component in the enhanced growth of immunized tobacco.

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