Relationships Among Carbohydrate Content of Kernels, Condition of Silks After Pollination, and the Response of Sweet Corn Inbred Lines to Infection of Kernels by *Fusarium moniliforme*

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ABSTRACT

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Twenty-two inbred lines were selected for carbohydrate analyses on the basis of a range of resistance to infection of kernels by *Fusarium moniliforme*. Relationships among the incidence of symptomatic and asymptomatic infection of kernels by *F. moniliforme* and the kernel content of individual sugars, total sugar, phytoglycogen, starch, and total carbohydrate were examined by linear regression analysis. Quantity and concentration of kernel carbohydrates were not related to infection of kernels by *F. moniliforme*. Asymptomatic infection of kernels by *F. moniliforme* was less for inbreds with silks that were green and actively growing at inoculation than for inbreds with green-brown or brown silks. Thus, browning and senescence of silks appeared to be important in initiating infection. Relationships among emergence of seedlings, carbohydrate variables, and infection of kernels by *F. moniliforme* were also examined by regression analyses, including principal factor regression. Infection of kernels by *F. moniliforme* and carbohydrate content of kernels were related to emergence of sweet corn inbred seedlings, yet much of the variation in emergence was not explained by these variables. Infection of kernels by *F. moniliforme* was the variable that was most highly related to emergence; however, symptomatic and asymptomatic infection accounted for only 39 and 30% of the variation in emergence, respectively. Many of the kernel carbohydrate variables were significantly related to emergence when the variation in emergence due to kernel infection was accounted for in multiple regression models. Based on these results, we do not expect that genetic improvement in the performance of sweet corn seedlings would be rapid if selection were based solely on the individual variables measured in this study.

The *shrunken-2* (*sh2*) endosperm mutation for high levels of sugars in kernels (16) has become widely used in the sweet corn (*Zea mays* L.) industry in recent years. *Shrunken-2* or "supersweet" sweet corn has broad consumer appeal because of its sweet taste and long refrigerator life. Unfortunately, cultivation of *sh2* corn has been plagued by poor emergence and poor seedling vigor (1,2,25,30). The high content of sucrose in kernels of *sh2* corn, which is accumulated at the expense of starch reserves (1,2,7, 16,27), is one of several factors associated with the poor vigor of *sh2* hybrids. Another factor affecting vigor is the infection of *sh2* kernels by fungal pathogens, especially *Fusarium moniliforme* J. Sheld. (teleomorph *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura) (5,9,26).

Emergence differs among and within sweet corn endosperm genotypes (*sh2, sugary-1* [*su*], and *sugary enhancer* [*se*]) and has been negatively correlated with incidence of *F. moniliforme* (5,9). It has been proposed that the high incidence of *F. moniliforme* in kernels of *sh2* genotypes results from the high content of kernel sugars, which may serve as a favorable substrate for pathogenic growth (25,30); however, several *su* genotypes with comparatively low levels of kernel sugars are susceptible to kernel infection by *F. moniliforme* (9).

Sweet corn inbreds that have good emergence and partial resistance to infection of kernels by *F. moniliforme* have been identified (9); however, the mechanisms of resistance have not been determined. Scott and King (23) observed that inheritance of resistance to ear rot caused by *F. moniliforme* in starchy (*Su*) corn is under maternal control and may be attributable to the genotype of the pericarp. They hypothesized that silk tissue could have been the site of gene action if the mode of kernel infection by *F. moniliforme* was through the silk as others had suggested (10,13,26). Headrick and Pataky (8) observed a similar maternal influence on the inheritance of resistance to infection of kernels by *F. moniliforme* in sweet corn. Similarly, Marsh and Payne (18) found that the incidence of kernel infection by *Aspergillus flavus* Link ex Fr. was affected by the color of silks after pollination, which was related to the moisture content of silks. The condition of the silks also may play a role in the resistance to kernel infection by *F. moniliforme*.

Several factors may affect the performance of sweet corn inbred seedlings. Closer examination of these factors may provide sweet corn breeders with a basis of selection for improved seedling vigor and emergence. The objectives of this study were to determine the relationships among carbohydrate content of kernels, condition of silks after pollination, and infection of sweet corn kernels by *F. moniliforme* and to determine the effects of these variables on the emergence of seedlings.

MATERIALS AND METHODS

Plant material. Twenty-two inbred lines were selected for carbohydrate analyses based on a range of resistance to infection of kernels by *F. moniliforme* (Table 1) (9). Most of the genotypes were *su* endosperm inbreds. Three *sh2* inbreds, two *se* inbreds, and one *Su* inbred were also included. Inbreds were planted in single rows of 15 plants each on 13 May 1987 and 17 May 1988 at the Agronomy/Plant Pathology South Farm, Urbana-Champaign, IL. Two replicates were performed in 1987 and four in 1988. When kernels matured, four self-pollinated ears from each row were harvested, dried with forced ambient air, and bulked by row. In addition, in 1988, a single ear from each row was harvested 21 days after pollination (i.e., the immature fresh-market stage), husked, frozen in liquid nitrogen, and stored at −20 C before carbohydrate analyses.

Incidence of *F. moniliforme*. All inbreds were inoculated and assessed for incidence of kernel infection by *F. moniliforme* as previously described (9). Symptomatic infection was determined as the percentage of randomly chosen kernels exhibiting signs or symptoms of *F. moniliforme*. Asymptomatic infection was determined as the percentage of healthy-appearing kernels (those with no signs or symptoms of infection or insect damage) exhibiting growth of *F. moniliforme* on a *Fusarium*-selective pentachloronitrobenzene medium (19).

Emergence. Emergence of seedlings was assessed as previously described (9). Emergence was based on uninoculated randomly chosen kernels of each inbred that were harvested the previous summer. Stand counts were made in the following spring for two replicates of two trials in which 60 kernels of each inbred were planted per replicate.

Carbohydrate analyses. The sugar content of mature dry kernels was determined in 1987. In 1988, sugar, phytoglycogen, and starch content of mature dry kernels and immature kernels were determined. Total sugar was calculated as the sum of the fructose, glucose, sucrose, and maltose fractions. Total carbohydrate was calculated as the sum of total sugar, phytoglycogen, and starch.

Sugars. Analysis of fructose, glucose, sucrose, and maltose contents of sweet corn kernels, which was previously described in detail (12), is outlined briefly. Mature dry kernels and immature freeze-dried kernels were ground into powder in a Wiley mill (Thomas Scientific Co., Philadelphia, PA) equipped with a 20-mesh screen. Sugars from each of the ground samples were dissolved four times with 1 ml 95% ethanol at 70 C, extracted following centrifugation, and brought up to 5 ml. A 150-μl sample of the total extract from each sample was evaporated in a reactivial. Fifty microliters each of STOX-oxime reagent and trimethylsiyl imidazole (Pierce Chemical Co., Rockford, IL) were added to each evaporated sample to form trimethylsilyl derivatives of sugars stabilized as oximes. The samples then were loaded onto a Hewlitt Packard model 5790A gas chromatograph. The peak areas for each trimethylsilyl sugar were expressed as proportions of the peak area of the internal standard and converted to milligrams of sugar per gram dry weight and milligrams of sugar per kernel by comparison with chromatograms from a set of premixed standards.

TABLE 1. Endosperm mutation and level of resistance to kernel infection by *Fusarium moniliforme* for inbred lines selected for carbohydrate analyses

Inbred	Endosperm[a]	Reaction to kernel infection by *F. moniliforme*[b]
IL125b	*su*	Resistant
IL776a	*se*	Resistant
IL773a	*su*	Resistant
IL781a	*su*	Resistant
I453	*su*	Intermediate
IL11d	*su*	Intermediate
IL27a	*su*	Intermediate
IL451b	*su*	Intermediate
IL784a	*sh2*	Intermediate
IL786a	*su*	Intermediate
IL789a	*su*	Intermediate
MA83610b	*se*	Intermediate
Oh43	*Su*	Intermediate
W6786	*su*	Intermediate
FA56a	*sh2*	Susceptible
I2123	*su*	Susceptible
I2256b	*su*	Susceptible
I5125	*su*	Susceptible
IL676a	*su*	Susceptible
IL783a	*sh2*	Susceptible
IL788a	*su*	Susceptible
MA83608b	*su*	Susceptible

[a] *su* = *sugary-1*, *SU* = starchy, *se* = *sugary enhancer*, and *sh2* = *shrunken-2*.
[b] Resistance classifications based on previous evaluations by Headrick and Pataky (9).

Phytoglycogen. Pellets from the sugar extractions were suspended in 1 ml of 10% ethanol overnight at 3 C to allow dissolution of the phytoglycogen (i.e., water-soluble polysaccharide component). The suspensions were clarified by centrifugation and dissolved three additional times in 1 ml of 10% ethanol at 70 C. The combined extracts were brought up to 5 ml (4). The phytoglycogen content of the extracts was determined from 20-μl samples by the phenol-sulfuric colorimetric method (11) with D-glucose as the standard. Absorbance was measured at 490 nm on a Beckman model DU-65 spectrophotometer.

Starch. Starch in the remaining pellets was determined by hydrolysis with amyloglucosidase (Boehringer Mannheim Biochemicals, Indianapolis, IN) (24). The pellets and enzyme were incubated on a shaker for 48 hr at 45 C in a sodium hydroxide and acetate buffer. The suspensions were clarified by centrifugation, and the pellets were rinsed twice with 1 ml of distilled water. Starch in the hydrolysate was quantified by the phenol-sulfuric colorimetric method (11) from 10-μl samples.

Statistical analysis of carbohydrates, infection, and emergence. Relationships among the incidence of symptomatic and asymptomatic infection of kernels by *F. moniliforme* and the kernel content and concentration of individual sugars, total sugar, phytoglycogen, starch, and total carbohydrate were examined. The effects of each of the carbohydrate variables and infection of kernels by *F. moniliforme* on seedling emergence were also examined. Means of kernel infection data collected from 1986–1988, and means of emergence data collected from 1987 and 1988 (9) were used in the analyses as the best estimates of the performance of inbreds. Significant, positive correlations between years were confirmed before combining data over years. Symptomatic infection in the hot, drought year of 1988 was not correlated with data from 1986 and 1987 because of a low incidence of *F. moniliforme* and confounding effects from high incidences of *Penicillium* and *Aspergillus* spp. Thus, these data were not included.

Correlation coefficients of symptomatic and asymptomatic infection and all carbohydrate variables were calculated. Relationships among individual carbohydrate fractions; the incidence of symptomatic and asymptomatic infection; and relationships among carbohydrates, infection, and emergence of seedlings were analyzed by simple least-squares linear regression. Carbohydrate fractions were analyzed both as concentrations (milligrams per gram dry weight) and on a per kernel basis (milligrams per kernel).

Stepwise multiple regression analysis (22) was then used to evaluate the effects of symptomatic and asymptomatic infection and the carbohydrate variables on emergence. Separate stepwise procedures were done for the two harvest times (mature dry kernels or immature kernels) and for the two units of measure for the carbohydrate fractions (concentration and content per kernel). Thus, the relationships among 10 independent variables (fructose, glucose, sucrose, maltose, total sugar, phytoglycogen, starch, total carbohydrate, symptomatic infection, and asymptomatic infection) and a dependent variable, emergence, were assessed. Multiple regression models from the stepwise procedure were chosen based on two criteria. First, the forward selection procedure was used, whereby independent variables were added to the model on the basis of the amount of variation explained (those variables accounting for the greatest increase in the coefficient of multiple determination, R^2). Variables included in the model by the forward selection procedure stayed in the model as new variables were added. Models with increasing numbers of variables were accepted until one or more of the variables in the model was not significant at the 0.10 level. Second, the MAXR procedure was used, whereby the model explaining the most variation for each number of independent variables was retained, and variables were interchanged as necessary to produce the highest R^2. The best MAXR model was determined by Mallow's C_p statistic, which approaches the number of variables in models that are appropriate (29). Model selection was not influenced by the significance of individual variables using this criterion.

Relationships among emergence of seedlings, carbohydrate

variables, and infection of kernels by *F. moniliforme* were also evaluated by principal factor regression. The principal factors among the 10 independent variables were determined by factor analysis using VARIMAX rotation to improve the interpretability of the solution (6,22). Variables were considered strongly loaded in a factor if the absolute value of the factor loading was greater than 0.50 (6). The number of factors retained was determined by Cattell's Scree Test (6) and by the ability of the factor to explain at least 10% of the total variation in the data. Principal factors were then used as independent variables in multiple regression analyses. Factors that explained at least 10% of the variation in emergence were retained in the principal factor regression model. The factor analysis was also performed with the dependent variable, emergence, included as a variable.

Growth of *F. moniliforme* on endosperm isolines. The effect of endosperm type on the growth and sporulation of *F. moniliforme* was tested by growing five isolates of the fungus on cornmeal agar (CMA) prepared from ground kernels of the inbred line IL451b that had *Su, su*, or *sh2* endosperm but otherwise were near-isogenic. CMA consisted of 4 g of cornmeal and 15 g of agar in 1 L of water. Fifteen replicates were performed of each isolate-endosperm combination. Radial growth was measured every 24 hr for 6 days. Sporulation was assessed 7 days after plating from three plates per endosperm per isolate. Microconidia were dislodged by gently rubbing the surface of a flooded plate with a glass rod and were then quantified using a hemacytometer. Data were subjected to analysis of variance.

Condition of silks. In 1987, the length and color of silks were assessed 4–7 days after pollination for 49 inbreds that were silk-inoculated with *F. moniliforme*. Silks were categorized for length (long, medium, or short), and for color (green, green-brown, or brown) based on degree of senescence. In 1988, the length and color of silks were assessed 4–7 and 18–21 days after pollination for 20 inbreds. Incidence of symptomatic and asymptomatic infection of kernels by *F. moniliforme* was determined as previously described (9) for each of the categories of silks. *T* tests were used to compare means of kernel infection by *F. moniliforme* for the different classes of silks.

Colonization of silks by *F. moniliforme* was followed in 1988 on inoculated and uninoculated silks of the resistant inbred IL125b and reciprocal crosses of IL125b with IL783a, a susceptible inbred. Silks were sampled weekly from emergence of silks to the mature dry kernel stage and plated on potato-dextrose agar amended with streptomycin and tetracycline and on pentachloronitrobenzene medium. Exposed silks (those that had grown beyond the husk) were cut from the ear. Husk-covered silks were excised from the tip, center, and butt of the ear with sterilized forceps after cutting through the husk with a sterilized scalpel (10). Kernels were plated as they became dry enough to be pulled from the cob. Anthers and pollen were also plated during anthesis.

TABLE 2. Means, standard deviations, and ranges of values for emergence, symptomatic and asymptomatic infection of kernels by *Fusarium moniliforme*, and carboyhydrate content of kernels for 22 sweet corn inbreds

Variable	Mean	Standard deviation	Range
Emergence (%)[a]	59	18.5	19–85
Symptomatic infection (%)[b]	17	20.3	1–79
Asymptomatic infection (%)[c]	35	24.3	2–80
Mature dry kernels (mg/g dry weight)			
Fructose	1.2	1.3	0.1–6.0
Glucose	1.9	1.5	0.2–7.0
Sucrose	23.2	5.3	12.0–32.7
Maltose	0.7	0.8	0–2.6
Total sugar	27.1	7.1	15.1–46.0
Phytoglycogen	120.4	80.7	0–298.9
Starch	306.7	90.8	184.8–586.5
Total carbohydrate	454.8	82.3	249.9–611.5
Mature dry kernels (mg/kernel)			
Fructose	0.15	0.12	0.01–0.48
Glucose	0.25	0.17	0.01–0.65
Sucrose	3.0	0.9	1.4–5.8
Maltose	0.08	0.10	0.00–0.37
Total sugar	3.5	1.1	1.5–6.8
Phytoglycogen	13.3	8.7	0.0–25.2
Starch	34.5	19.1	13.0–90.5
Total carbohydrate	51.1	21.3	17.4–94.8
Immature kernels (mg/g dry weight)			
Fructose	12.9	5.7	8.0–31.8
Glucose	14.3	5.1	8.4–30.7
Sucrose	56.3	33.6	27.5–159.6
Maltose	2.0	1.5	0.4–6.3
Total sugar	85.5	39.2	48.9–188.8
Phytoglycogen	185.9	75.5	35.9–308.4
Starch	256.7	102.1	108.4–491.1
Total carbohydrate	528.1	45.9	458.1–596.9
Immature kernels (mg/kernel)			
Fructose	0.9	0.4	0.6–2.1
Glucose	1.0	0.4	0.7–2.0
Sucrose	3.8	1.6	1.8–8.6
Maltose	0.14	0.11	0.02–0.43
Total sugar	5.8	1.8	3.1–10.2
Phytoglycogen	13.8	6.1	2.5–23.3
Starch	19.4	9.3	7.1–38.1
Total carbohydrate	39.1	9.6	24.7–67.0

[a] Emergence evaluated in the spring for kernels produced the previous summer.
[b] Symptomatic infection = percentage of kernels exhibiting signs or symptoms of *F. moniliforme*.
[c] Asymptomatic infection = percentage of healthy-appearing kernels exhibiting growth of *F. moniliforme* on pentachloronitrobenzene medium.

RESULTS

The 22 inbreds tested exhibited a wide range of responses for emergence, infection of kernels by *F. moniliforme*, and carbohydrate content of kernels (Table 2).

Relationship among carbohydrates, infection of kernels by *F. moniliforme*, and growth of *F. moniliforme* on endosperm isolines. Kernel carbohydrates were not significantly related to infection of kernels by *F. moniliforme*. Regressions of incidence of symptomatic and asymptomatic infection on individual carbohydrate fractions and on total sugars or total carbohydrates were not significant.

Growth and sporulation of five isolates of *F. moniliforme* did not differ on CMA prepared from *Su, su*, and *sh2* near-isogenic lines. Growth on CMA prepared from the *su* endosperm line was initially greater than on CMA prepared from the other endosperm types, but within 3 days radial growth of *F. moniliforme* was equal on all media.

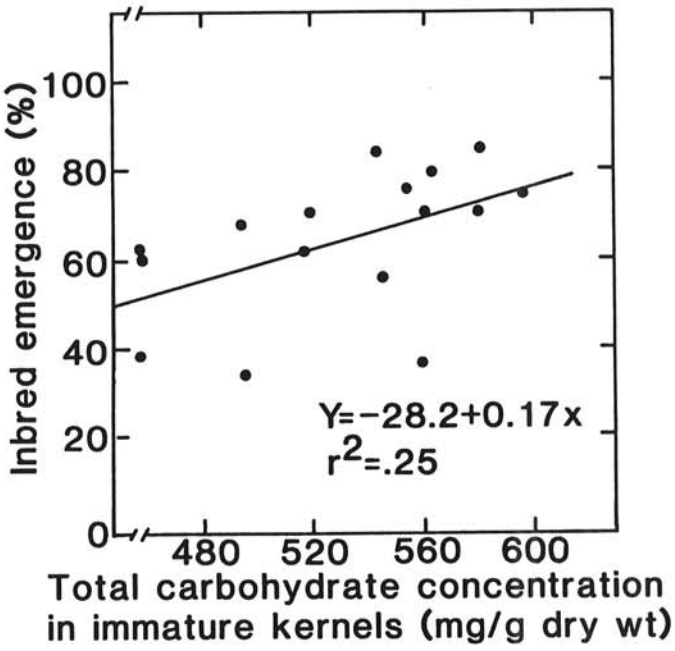

Fig. 1. Emergence of sweet corn inbreds and total carbohydrate concentration of immature kernels.

Relationships among carbohydrates, infection by *F. moniliforme*, and emergence. Total concentration of carbohydrates in immature kernels was the only individual carbohydrate variable to be significantly related to emergence. Emergence increased as the carbohydrate content in the immature kernels increased (Fig. 1). Total carbohydrate content in mature dry kernels was not significantly related to emergence.

Symptomatic infection of kernels by *F. moniliforme* was significantly related to emergence and explained 39% of the variation in emergence ($r^2 = 0.39$) (Table 3). Asymptomatic infection was also significantly related to emergence, although asymptomatic infection explained only 30% of the variation in emergence. The amount of variation in emergence explained by symptomatic infection and various carbohydrate variables was often greater than the sum of their individual effects, since the strength of the relationship between emergence and the carbohydrate variable was increased when adjusted for variation in infection, and vice versa.

Stepwise multiple regression of emergence on infection and carbohydrate variables resulted in models with two to four variables, depending on the selection criterion (Table 3). The forward selection criterion resulted in two variable models that included symptomatic infection and a carbohydrate variable. Symptomatic infection and total sugar concentration in mature dry kernels explained 52% of the variation in emergence. Symptomatic infection and total carbohydrate concentration in immature kernels explained 65% of the variation in emergence (Table 3). The MAXR selection criterion resulted in models with four variables, and higher R^2 values. Symptomatic infection and fructose, glucose, and maltose concentrations in mature dry kernels explained 68% of the variation in emergence. Symptomatic infection, maltose concentration, total sugar concentration, and total carbohydrate concentration in immature kernels explained 79% of the variation in emergence (Table 3).

Principal factor analysis using VARIMAX rotation produced three or four principal factors that accounted for more than 85% of the variation among the 10 independent variables (Table 4). For the principal factor analysis of carbohydrate fractions of mature dry kernels and infection of kernels by *F. moniliforme*, factor one was a sugar/starch factor, consisting of sucrose, maltose, total sugar, starch, and total carbohydrate, which explained 33% of the variation among the independent variables. Factor two was a fructose/glucose factor, consisting of fructose, glucose, and total sugar, which accounted for an additional 24% of the variation. Factor three was an infection factor consisting of symptomatic and asymptomatic infection of kernels by *F. moniliforme*, which accounted for an additional 20% of the variation.

TABLE 3. Summary of regression models to explain variables affecting emergence of sweet corn inbreds

	Mature dry kernels[a]			Immature kernels[a]		
Procedure	Variables included in model	Prob. > F for factor	R^2 model	Variables included in model	Prob. > F for factor	R^2 model
Linear[b]	Symptomatic infection	0.007	0.39	...	...	...
Stepwise						
Forward[c]	Symptomatic infection	0.002	0.52	Symptomatic infection	0.001	0.65
	Total sugar	0.068		Total carboyhydrate	0.016	
MAXR[d]	Symptomatic infection	0.021	0.68	Symptomatic infection	0.001	0.79
	Fructose	0.055		Maltose	0.050	
	Glucose	0.018		Total sugar	0.028	
	Maltose	0.031		Total carbohydrate	0.005	
Principal factors[e]						
	Factor 2 (fructose/glucose)	0.173	0.47	Factor 2 (starch)	0.146	0.46
	Factor 3 (infection)	0.007		Factor 3 (infection)	0.011	

[a] Carbohydrate content expressed as mg/g dry weight.
[b] Best linear model. Symptomatic infection determined for mature dry kernels only.
[c] Models selected on the basis of the forward selection procedure.
[d] Models selected on the basis of the MAXR selection procedure and Mallow's statistic, C_p.
[e] Determined by principal factor analysis using VARIMAX rotation.

Factor four consisted of phytoglycogen and starch and accounted for an additional 15% of the variation (Table 4). Thus, the four principal factors accounted for 92% of the total variation among the 10 independent variables. For the principal factor analysis of carbohydrate fractions of immature kernels and infection kernels by *F. moniliforme*, factor one was a sugar factor, consisting of fructose, glucose, sucrose, maltose, and total sugar, which explained 40% of the variation among the independent variables. Factor two was a starch factor, consisting of starch, total carbohydrate, and phytoglycogen, which accounted for an additional 25% of the variation. Factor three was an infection factor, consisting of asymptomatic and symptomatic infection of kernels by *F. moniliforme*, which explained an additional 21% of the variation (Table 4). Thus, the three principal factors accounted for 86% of the total variation among the 10 independent variables.

When emergence was included in the principal factor analysis, it loaded strongly with the *F. moniliforme* infection factor (factor three in both analyses) and weakly with one of the carbohydrate factors. For mature dry kernels, the factor loadings for emergence were −0.80 and −0.32 in the *F. moniliforme* infection factor and the fructose/glucose factor, respectively. For immature kernels, the factor loadings for emergence were −0.88 and 0.20 in the *F. moniliforme* infection factor and the starch factor, respectively.

Principal factor regression using the factors as independent variables confirmed the association of emergence and infection. Infection of kernels by *F. moniliforme* was the factor of primary importance in explaining emergence. For mature dry kernels, the infection factor explained 37% of the variation in emergence, and the fructose/glucose factor explained an additional 10% of the variation. The sugar/starch and phytoglycogen factors did not make significant contributions to the model. For immature kernels, the infection factor explained 36% of the variation in emergence, and the starch factor explained an additional 10% of the variation. The sugar factor did not make a significant contribution to the model (Table 3).

Condition of silks. The condition of exposed silks, as assessed by color at the time of inoculation, was related to the incidence of symptomatic and asymptomatic infection of kernels by *F. moniliforme*. Symptomatic infection was greatest when silks were green-brown at the time of the first inoculation (Table 5). In 1987, symptomatic infection was least among kernels from ears with green silks. Symptomatic infection was least when brown silks were inoculated in 1988; however, only three observations of brown silks were made at the first inoculation in 1988, because silks stayed green for an extended period of time due to poor pollination under hot, dry conditions. Symptomatic infection was

TABLE 4. Varimax-rotated factor loadings and cumulative variance accounted for by the principal factors of independent variables for mature dry and immature inbred corn kernels

Variable	Factor 1[a]	Factor 2	Factor 3	Factor 4
Mature dry kernels				
Symptomatic infection	−0.08	−0.01	0.97*	0.05
Asymptomatic infection	0.05	−0.23	0.91*	0.15
Fructose[b]	0.25	0.92*	−0.21	−0.01
Glucose	−0.01	0.95*	−0.02	0.07
Sucrose	0.85*[c]	0.28	−0.20	0.24
Maltose	0.78*	0.27	−0.04	−0.27
Total sugar	0.73*	0.61*	−0.19	0.15
Phytoglycogen	−0.06	0.08	0.15	0.97*
Starch	−0.79*	0.05	−0.21	−0.52*
Total carbohydrate	−0.87*	0.18	−0.10	0.34
Cumulative variance accounted for (%)	33.2	56.9	76.7	91.8
Immature kernels				
Symptomatic infection	−0.02	−0.05	0.94*	...
Asymptomatic infection	−0.22	−0.24	0.90*	...
Fructose	0.86*	−0.16	−0.31	...
Glucose	0.88*	−0.16	−0.29	...
Sucrose	0.84*	0.00	0.18	...
Maltose	0.76*	−0.42	−0.40	...
Total sugar	0.97*	−0.08	−0.01	...
Phytoglycogen	−0.16	−0.85*	0.24	...
Starch	−0.29	0.93*	−0.14	...
Total carbohydrate	−0.30	0.78*	0.06	...
Cumulative variance accounted for (%)	39.8	64.7	86.3	...

[a] Factor procedure with VARIMAX rotation (21). Factors retained on the basis of Cattell's Scree Test (6) and the amount of variation explained.
[b] All carbohydrate fractions expressed as mg/g dry weight.
[c] * = variables with factor loadings of absolute value greater than 0.50 were considered to be strongly loaded in the factor (6).

TABLE 5. The effect of postpollination silk condition on incidence of symptomatic and asymptomatic infection of kernels by *Fusarium moniliforme* from inoculated ears of inbred corn

	1987			1988					
	1 wk postpollination			1 wk postpollination			3 wk postpollination		
Silk color	*n*[a]	Symptomatic infection[b]	Asymptomatic infection[c]	*n*	Symptomatic infection	Asymptomatic infection	*n*	Symptomatic infection	Asymptomatic infection
Brown	18	9.5	66.7	3	5.3*[d]	26.7	46	14.6	20.4*
Green-brown	32	11.5	66.1	28	18.8	18.4	23	14.0	11.8
Green	44	6.1*	42.4*	39	11.6	15.7	0	...	...

[a] Number of inbred lines observed in each category.
[b] Symptomatic infection = percentage of kernels exhibiting signs or symptoms of *F. moniliforme* infection.
[c] Asymptomatic infection = percentage of healthy-appearing kernels exhibiting growth of *F. moniliforme* on pentachloronitrobenzene medium.
[d] * = percentage of kernel infection significantly different (at the 0.10 level) from the percentage of kernel infection of kernels from ears with green-brown silks.

also confounded in 1988 by high incidences of *Penicillium* and *Aspergillus* spp. Asymptomatic infection of kernels was greatest when silks were brown at the first inoculation and was least when silks were green at the first inoculation, although this difference was not significant in 1988, when asymptomatic infection was substantially lower. When silks remained green-brown compared to those that turned brown by the second inoculation in 1988, asymptomatic infection was less. None of the silks remained green 3 wk after pollination.

Colonization of silks by *F. moniliforme* was related to conditions of silks. Little colonization occurred until the exposed silks began to senesce, and little growth of the fungus into the ear was observed until the silks covered by husks began to senesce (Table 6). Silks were colonized by *F. moniliforme* from the tip of the ear downward, and in only one instance was *F. moniliforme* isolated from silks at the butt of the ear (from IL125b × IL783a on 1 Sept). Inoculated ears exhibited a slightly greater incidence of *F. moniliforme* than uninoculated ears, and *F. moniliforme* was isolated further down the silk of inoculated ears. In the last week of collection, incidence of *F. moniliforme* on silks dropped dramatically, including 0% on IL125b. At this time, *F. moniliforme* was recovered from kernels.

DISCUSSION

None of the individual carbohydrate fractions of kernels were related to infection by *F. moniliforme* in this study. Previously, it had been suggested that the high sucrose content of *sh2* kernels increased infection by *F. moniliforme* (1,25). Although no specific information is available on carbon utilization by *F. moniliforme*, related species (e.g. *F. oxysporum* Schlectend. ex Fr.) are able to effectively use a wide range of carbon sources, including sugars and starch (31). Thus, we might expect that *F. moniliforme* could use many carbon sources, based on its wide host range and outstanding saprophytic ability. Therefore, it is not surprising that carbohydrate composition of kernels was not related to infection of kernels by *F. moniliforme*.

The condition of silks exposed to inoculation was related to infection of kernels by *F. moniliforme*, a fungus described as only weakly pathogenic (14). Inbreds in which silks were green and actively growing when inoculated had less asymptomatic infection of kernels than those in which silks were green-brown or brown and beginning to senesce. Thus, brown, senescent silks appeared to be important in initiating infection, presumably because of the competitive saprophytic ability of *F. moniliforme*. However, it was not obvious why symptomatic infection by *F. moniliforme* was highest when green-brown silks were inoculated and asymptomatic infection was highest when brown silks were inoculated. Moisture content of the silks and time of kernel infection may have been important factors. When green-brown silks were inoculated with *F. moniliforme*, the fungus may have rapidly grown on the silks and colonized the surface of developing kernels without moisture limitations. Following surface colonization but before senescence of silks, the fungus may have entered the kernel at the juncture between the silk and kernel or in the tip cap region (13,21). Entrance of *F. moniliforme* under ideal conditions early in kernel development may have promoted mycelial growth and sporulation, leading to symptom development on kernels. Conversely, brown silks may have been limited in moisture, resulting in slowed fungal growth, a lack of extensive surface colonization, and inhibition of development of symptoms on kernels. In addition, a decrease in kernel moisture late in the season may have promoted asymptomatic rather than symptomatic infection of kernels. Koehler (13) observed a substantial increase in the percentage of sound kernels infected by *F. moniliforme* after kernel moisture had dropped below 34%. Marsh and Payne (18) observed a similar pattern of kernel infection by *A. flavus*; however, they found less kernel infection when brown silks were inoculated. Likewise, Styer and Cantliffe (25) reported that infection of kernels by *F. moniliforme* was greater when silks were inoculated 10 days after pollination than when inoculations were made at later growth stages, even though their inoculum suspension was injected into silks at the ear tip rather than sprayed onto exposed silks. We have observed that *F. moniliforme* did not survive on silks under the husk after the husk-covered silks senesced, which supports the hypothesis that moisture may limit the growth of *F. moniliforme* on silks. The placement of plastic bags on the inoculated silks in our experiments may have resulted in an artificial inflation of the incidence of *F. moniliforme* on brown silks compared to green-brown silks, if, in fact, moisture was a limiting factor in silk colonization. Nevertheless, green silks, which were high in moisture, appeared to be associated with resistance to colonization of silks by *F. moniliforme*.

TABLE 6. Isolation of *Fusarium moniliforme* from inoculated silks of three sweet corn genotypes sampled at weekly intervals in 1988[a]

Collection date	Genotype[c]	Silk condition	Number of silk pieces[b]		
			Exposed	Tip	Center
7-27	IL125b	None	...	...	...
	IL125b × IL783a	Green	0	0	0
	IL783a × IL125b	Green	0	0	0
8-3	IL125b	Green	0	0	0
	IL125b × IL783a	Green	1	0	0
	IL783a × IL125b	Green	0	0	0
8-10	IL125b	Green	...	...	...
	IL125b × IL783a	Exposed silks senescing	1	0	0
	IL783a × IL125b	Exposed silks senescing	4	0	1
8-17	IL125b	Exposed silks senescing	4	0	0
	IL125b × IL783a	Exposed silks brown	5	1	0
	IL783a × IL125b	Exposed silks brown	5	0	0
8-24	IL125b	Exposed silks brown	5	0	0
	IL125b × IL783a	Exposed silks brown	5	2	0
	IL783a × IL125b	Husked silks senescing	5	5	1
9-1	IL125b	Husked silks senescing	5	1	0
	IL125b × IL783a	Husked silks senescing	5	5	5[d]
	IL783a × IL125b	Husked silks senescing	5	5	1
9-8	IL125b	Husked silks dry	0	0	0
	IL125b × IL783a	Husked silks dry	5	4	2
	IL783a × IL125b	Husked silks dry	3	0	0

[a] Silks were inoculated with *F. moniliforme* 1 and 3 wk postpollination. F_1 hybrids inoculated 8-5 and 8-19. IL125b inoculated 8-11 and 8-26.
[b] Five silk pieces from each ear were plated on pentachloronitrobenzene medium.
[c] All plants were self-pollinated by hand. F_1 hybrids pollinated 7-28. IL125b pollinated 8-2.
[d] *F. moniliforme* was also isolated from five pieces of silk collected from the butt end of the ear.

The silk sampling experiment confirmed earlier reports (10,13,17,26) that *F. moniliforme* enters ears through silks. Infected kernels were recovered both before and after the husk-covered silks senesced, confirming that *F. moniliforme* may enter kernels before complete senescence of silks and survive in mature kernels after the silk has deteriorated.

Infection of kernels by *F. moniliforme* was the variable most closely associated with emergence of sweet corn inbreds in this study; however, symptomatic and asymptomatic infection of kernels accounted for just 39 and 30% of the variation in emergence, respectively. In a related study in which 115 genotypes were evaluated (9), symptomatic and asymptomatic infection of kernels significantly affected emergence, yet accounted for less than 10% of the variation. Thus, although infection of kernels by *F. moniliforme* had a significant negative effect on the emergence of sweet corn inbreds, other unidentified variables accounted for a much greater portion of this variation. These results corroborate the conclusion of Styer and Cantliffe (25) that infection by *F. moniliforme* contributed to the poor seedling vigor of *sh2* genotypes but did not appear to be the primary cause.

Kernel carbohydrate fractions were not individually related to emergence. Hannah and Cantliffe (7) also found that sugar content alone did not account for differences in emergence among sweet corn hybrids. Wann et al (27,28) determined that total carbohydrate content differed very little among sweet corn cultivars of different endosperm types that exhibited wide variation in seedling vigor. Hannah and Cantliffe (7) found that the emergence of sweet corn with the *brittle-A* (*bt-A*) endosperm mutation was much better than that of *sh2* genotypes, although the sugar content of the kernels was nearly equal. Therefore, the poor emergence of sweet corn lines with the *sh2* endosperm type does not seem solely attributable to the high sugar content of the kernels but may be an inherent problem of this endosperm type, which is pleiotropic to the effect of this allele on kernel sugar content. These effects require further investigation.

Although kernel sugar and starch contents individually were not significantly related to emergence in this study, many of the carbohydrate variables were significantly related to emergence when the variation in emergence due to infection by *F. moniliforme* was accounted for in multiple regression models. The effects of carbohydrates on emergence may be due to induced changes in fungal metabolism, perhaps including regulation of enzymes and/or toxins that inhibit germination and seedling growth (31). Carbohydrates also may have a direct effect on seedling vigor by providing energy reserves.

Relationships among carbohydrate contents of kernels and emergence were also evident by principal factor regression analysis. The principal factors model for mature dry kernels explained 47% of the variation in emergence. The *F. moniliforme* infection factor explained the largest portion of that variation (37%), as was expected based on linear regression analysis and previous results (9). The fructose/glucose factor accounted for 10% of the variation in emergence. The importance of the fructose/glucose factor, which was inversely related to emergence, is more difficult to explain. Prasad and Chaudhary (20) found that mycelial growth and spore formation of *F. oxysporum* f. sp. *udum* were influenced by the utilization of fructose and glucose, but not sucrose. Woltz and Jones (31) reported that glucose may be important in regulating pectolytic enzyme activity of some *Fusarium* spp. Brown (3) observed that glucose in culture media reduced variation among *Fusarium* spp. Lampe (15) found that reducing sugars were present early in kernel development and were restricted to the base of the kernel in older kernels. The possible effects of these observations on emergence of seedlings are unclear, since none of the sugars were individually related to emergence or infection of kernels by *F. moniliforme*.

The value of principal factor analysis lies in the identification of related variables within a data set and may provide insight into the effects of related variables. For example, in our data sets, 10 independent kernel characteristics were explained by three to four principal factors, including a *F. moniliforme* infection factor, a sugar factor, and a starch/total carbohydrate factor or a sugar/starch factor. The biological basis of these factors is evident. Sugar and starch are inversely related due to the gene action of the endosperm mutations. Also, infection was not affected by carbohydrate content; thus, variation caused by the two infection variables would not be expected to be explained by a carbohydrate factor. Many of the relationships between individual variables could be predicted from correlation matrices; however, the principal factor analysis provides a basis for grouping multiple, correlated variables into factors that can improve the interpretability of these relationships.

The models derived from our data using stepwise and principal factor regression procedures are analytical and applicable only to the data sets from which they were derived. They are not meant to be predictive. Nonetheless, exploratory procedures such as these may have greater value than traditional procedures (such as simple regression and correlation) when examining problems of complex etiology, such as poor emergence. In this study, multiple regression models were derived that explained a greater portion of the variation in emergence than did simple regression models. The multiple regression models, however, were based on single variables that in themselves are often of unknown biological importance. The factor analysis resulted in the grouping of biologically related variables, thus providing the basis for a more meaningful interpretation of the regression model. The regression models examined in this study resulted in an appreciable amount of variation in emergence that was not accounted for by infection with *F. moniliforme* and carbohydrate content of kernels. It is likely, therefore, that with a larger inbred population and more independent variables (e.g., pericarp thickness, electrolyte leakage, C:N ratios, insect populations, seed treatment fungicides, etc.), a more accurate and comprehensive model for emergence of sweet corn seedlings may be derived using such exploratory analytical techniques.

The results reported here indicate that selection for delayed senescence of silks may reduce the incidence of kernel infection by *F. moniliforme* and, therefore, may improve emergence of seedlings. Green silks (resistance) in the female inbred to be used in a resistant × susceptible cross could be especially important, since the silks are maternal tissue. We have found that infection of kernels by *F. moniliforme* and kernel carbohydrate content affect emergence of sweet corn inbreds, yet much of the variation in emergence is not explained by these variables. Thus, genetic improvement in the emergence and performance of seedlings would not be expected to be rapid if selection is based solely on the variables measured in this study. Conversely, direct selection for improved seedling vigor would be expected to result in greater gains in performance of seedlings but would not be expected to result in improved resistance to infection of kernels by *F. moniliforme*.

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