A Mathematical Simulation of Growth of *Fusarium* in Maize Ears After Artificial Inoculation

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ABSTRACT

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Fusarium spp. in maize can contaminate the grain with mycotoxins if environmental conditions are favorable for fungal growth. To quantify the relationship between growth of *Fusarium* spp. and environmental conditions, a mathematical model was developed to simulate growth of *F. graminearum* and *F. verticillioides* on maize ears following silk inoculation in field experiments from 1992 to 1995. Each species was inoculated separately and as a mixture of the two for 3 of the 4 years on one maize hybrid. Disease progress in ears was measured by a visual rating scale that was converted to percent visual infection. Measurements were made at regular time intervals after silks were inoculated 5 days after silk emergence. Differential equations were used to relate growth rates of *Fusarium* spp. in maize ears to hourly air temperature and relative hu-

midity and to daily precipitation. Integration of these equations over time produced quantitative estimates of fungal growth. Model calculations compared well with measurements ($R^2 = 0.931$, standard error of estimate [SEE] = 2.11%) of percent visual disease infection of maize ears over 3 years. The model was tested against a second set of data ($R^2 = 0.89$, SEE = 5.9%) in which silks were inoculated at nine different times after first silk emergence for each of 2 years (1994 and 1995) with the two species of fungi on the same maize hybrid. At this time, a silk function was developed to account for changes in the susceptibility of silks to disease. *F. graminearum* responded to wet conditions more than *F. verticillioides*, and for the conditions of this experiment, grew much faster than *F. verticillioides* when inoculated separately. When they were inoculated together, *F. graminearum* growth rates were much lower, indicating some interference by *F. verticillioides*. During 1993, weather conditions before inoculation reduced the growth of both species in silks.

Growth of *Fusarium* in ears of maize (corn, *Zea mays* L.) is an important concern for maize production due to a negative effect on yield and grain quality. These fungal species produce mycotoxins that can adversely affect growth rates of livestock fed contaminated maize (8,11). In Canada, the toxins of most concern are deoxynivalenol (DON, vomitoxin) and zearalenone, produced by *F. graminearum* Schwabe [teleomorph = *Gibberella zeae* (Schwein.) Petch] followed by fumonisin B₁, produced by *F. verticillioides* (Sacc.) Nirenberg [= *F. moniliforme*, teleomorph = *G. fujikuroi* (Sawada) Wr.] (21). These fungi can infect ears through wounds caused by birds and insects or from colonization of silks and growth through the silk channels to the kernels (19,20). In years of severe infection by *F. graminearum*, the most common route is through the silks (21).

Year-to-year variation in amounts of disease is highly dependent on weather conditions although the response to weather varies among species. For example, *F. graminearum* grows better in wet years than *F. verticillioides* (8,20). Quantification of weather effects on growth of *Fusarium*, measured as disease development by linear regression analysis, was done by Moschini and Fortugno (9) for wheat and Vigier et al. (21) for maize. A mechanistic model of mold growth of *Aspergillus flavus* Link ex Fries mold growth based on differential equations was developed by Pitt (10). However, this model simulated fungal growth only under controlled environmental conditions. Various types of simulation

models of diseases for field conditions have been developed. Simulators such as EPIMAY (22) for southern corn leaf blight were cited by Campbell and Madden (4). To our knowledge, there has been no such model for *Fusarium* spp. on maize.

The objective of this study was to develop a mechanistic or conceptual model of *Fusarium* growth in maize ears under field conditions after artificial inoculation with two species of *Fusarium* (*F. graminearum* and *F. verticillioides*), separately and together, so that the amounts of *Fusarium* percent visual infection, alone and in competition, could be estimated from weather parameters. This is a first step in building a general model for calculating levels of toxins over regions based on weather parameters.

THEORY AND APPROACHES

Model description. Many processes can best be described by how they change with time using a differential equation. In this study, the model was based on a differential equation relating rate of growth of a single species to amount of growth (G), a temperature function (T_F), and an ear wetness function (W_F). The potential for exponential growth was made possible by making the rate of growth directly proportional to the amount of growth. That is

$$dG/dt = a_1 G T_F W_F \quad (1)$$

The amount of growth (G) is determined by visual inspection of maize ears and is expressed as the percentage of the ear that is visually infected by the fungi being studied. Therefore, G will be referred to as percent visual infection. More detail on how G was

measured is provided below. The temperature function was an Arrhenius-like equation from Pitt (10):

$$T_F = A \exp \left[- \left(\frac{\alpha^2}{(T - T_{\min})} + \frac{\beta^2}{(T - T_{\max})} \right) \right] \quad T_{\min} < T < T_{\max} \quad (2)$$

where T is hourly temperature (either silk or air, degree Celsius), $T_{\min}$ is a minimum temperature (degree Celsius) for growth, $T_{\max}$ is a maximum temperature (degree Celsius) for growth, α and β are shape factors (degree Celsius to the one-half [$^{\circ}\text{C}^{1/2}$]), and A is a scaling factor (dimensionless). All shape factors and other coefficients are listed with units in Table 1. T_F is set to zero for temperatures less than $T_{\min}$ and greater than $T_{\max}$. An optimum temperature was defined by setting the differential of equation 2 to zero when T was equal to the optimum temperature (T_{opt}) (10). By rearranging, β is expressed as

$$\beta = \alpha(T_{\max} - T_{\text{opt}})/(T_{\text{opt}} - T_{\min}) \quad (3)$$

The scaling factor (A) was calculated by setting T_F equal to 1.0 when T was equal to T_{opt} for *F. graminearum* and T_F was equal to 1.08 for *F. verticillioides*. The slightly higher value of 1.08 corresponded to a higher growth rate at higher temperatures for *F. verticillioides* as explained below. This left only α , $T_{\max}$, $T_{\min}$, and T_{opt} as unknowns, but values of these coefficients will determine the shape of the temperature response function.

Values for these coefficients were difficult to determine. The data on growth rates from Reid et al. (15) indicated values of 28 and 31°C for T_{opt} and $T_{\max}$ for *F. graminearum*, respectively. Values of T_{opt} and $T_{\max}$ for *F. verticillioides* were set at 31 and 35°C, respectively, to correspond with evidence that *F. verticillioides* grows faster at higher temperatures (17). The function for *F. verticillioides* was multiplied by a factor of 1.08 so that the two species had similar temperature response functions for temperatures below 28°C as indicated by the data from Reid et al. (15). Finally, α and $T_{\min}$ were set at 3 and 15°C, respectively, to correspond to Alberts et al. (1), who showed considerable growth of *F. verticillioides* at 20°C, and the temperature response functions of Marin et al. (6), with growth as low as 10°C depending on water availability. The temperature response function will be discussed in more detail below.

The ear wetness function was a modification of the model of Rao et al. (12) in which a film of water (R) was assumed to adhere to the surface of the ear. Rao et al. (12) assumed a maximum thickness of 0.8 mm, which we increased to 1.0 mm to allow for some of the water to occur in the husks and the husk–kernel interface. It is the water status of the kernels not the sheath surface that will influence the spread of the fungi. Precipitation (P) is the rainfall or irrigation in millimeters of water. Changes in the thickness of this water film was expressed mathematically by

$$\Delta R = -a_2(V_{ps} - V_p) + P \quad (4)$$

where ΔR is the change in thickness of the film during a time step in the model (in this case 1 h), V_p is the vapor pressure, and V_{ps} is the saturated vapor pressure. More detail on time steps is given below. The equation $V_{ps} - V_p$ is the vapor pressure gradient and was calculated from relative humidity and air temperature. The film thickness was never less than zero or greater than the maximum value of 1 mm. The coefficient a_2 was a function of the physical dimensions of the ear and the thickness of the ear-atmosphere boundary layer. The latter was, in turn, related to the wind speed at ear height in the maize canopy (12). In addition, a_2 was related to the water diffusion properties of the maize ear husk. Because wind speed at the ears was not available in these experiments, is seldom measured in general, and very little is known on water transfer properties of husks, a simple coefficient was assumed

that represented average wind conditions and ear characteristics. The vapor pressure deficit was assumed to be the main environmental variable controlling water loss from the ear. The wetness function (W_F) was expressed as

$$W_F = (1 + a_3R) \quad (5)$$

where a_3 is an empirical coefficient.

The differential equations were solved by using simple trapezoidal integration. For example, equation 1 became

$$G_i = G_E + \sum_{i=n}^N a_1 G_{i-1} T_F W_F \Delta t \quad (6)$$

That is, we started with an initial small value G_E and stepped through the growth period 1 h at a time. The subscript i represents the number of hours after inoculation, Δt is the time step of 1 h, n is the time when the fungi reached the kernels from the inoculation site in the silks, and N is the total number of hours from inoculation to the end of the growth period. However, in order to determine G_E and n , the following equation was needed to describe growth of the fungi down the silk channels:

$$dG/dt = c_1 a_5 T_F W_F S_F \quad (7)$$

where c_1 is an empirical coefficient, a_5 is a coefficient to describe the inhibiting effects of weather on silk growth before inoculation, and S_F is a silk function to account for silk aging effects as described by Reid et al. (13). The a_5 coefficient is related to precipitation deficits (precipitation – potential evapotranspiration) for a 40-day period before inoculation. Potential evapotranspiration was calculated from maximum and minimum air temperatures using the equations of Baier and Robertson (3) and

TABLE 1. List of coefficients which appear in the derivation of the model in the text with values, standard errors in brackets and unit^a

Coefficient	Name	Values (SE)
a_1	Ear growth	0.0243 (0.00516) h ⁻¹
a_2	Ear air exchange	0.000305 (0.000013) mm/kPa
a_3 (Gr)	Wetness response	1.710 (0.545) mm ⁻¹
a_3 (Ve)	Wetness response	0.930 (0.410) mm ⁻¹
a_5 1993	Pre-silk water stress	0.1918 (0.0098)
a_5 1992	Pre-silk water stress	1.0
a_5 1994	Pre-silk water stress	1.0
b_1	First order toxin	3.66 (0.540) µg/g/%
b_2	Second order toxin	0.0253 (0.00916) µg/g/% ²
c_1	Silk growth coefficient	0.00413%/h
c_2	Silk interference coefficient	0.500% ⁻¹
c_3	Ear interference coefficient	0.316% ⁻¹
d_1	Silk age y-intercept	-0.25
d_2	Silk age 1st critical level	35 MGDD
d_3	Silk age 2nd critical level	55 MGDD
d_4	Silk age slope	0.050 (0.017) MGDD ⁻¹
d_5	Silk age plateau factor	0.20
$T_{\max}$ (Gr)	Maximum temperature for fungal growth	31°C
$T_{\max}$ (Ve)	Maximum temperature for fungal growth	35°C
T_{opt} (Gr)	Optimum temperature for fungal growth	28°C
T_{opt} (Ve)	Optimum temperature for fungal growth	31°C
A (Gr)	Scale factor (temperature function)	1.00
A (Ve)	Scale factor (temperature function)	1.08
$T_{\min}$	Minimum temperature	15°C
α	Shape factor (temperature function)	3°C ^{1/2}
G_0	Initial percent infection in silks	0.3%
G_E	Initial percent infection in ears	0.6%

^a Coefficients are dimensionless when no units are shown. Gr = *Fusarium graminearum*, Ve = *F. verticillioides*.

Baier (2). The silk function (dimensionless) was assumed to be equal to one for conditions of experiment A when inoculation took place 5 days after silking. We will return to the silk function when describing how the model was compared with results from experiment B. Solving equation 7 by simple trapezoidal integration resulted in

$$G_i = G_0 + \sum_{j=1}^n c_1 a_3 T_F W_F S_F \Delta t \quad (8)$$

Therefore, during the silk phase, percent infection increased from an initial value of G_0 to a value of G_E in n days. G_0 will correspond to the amount of inoculum.

In this study, growth (G_1 and G_2) of two species were studied. Therefore, two sets of equations were used that differed only in the temperature function and the value of the a_3 (wetness) coefficient. There was also a mixed inoculum that involved interference of one organism with the other. The assumption was made that *F. verticillioides* (G_2) interfered with the growth of *F. graminearum* (G_1) based on the results of Reid et al. (15). Therefore, for the case when inoculum was mixed, equations for the two species were altered as follows. For *F. graminearum* with mixed inoculum, the following two equations replaced equations 6 and 8, respectively,

$$G_{1i} = G_E + \sum_{j=n}^N a_1 G_{1(i-1)} T_F W_F (1 - c_2 G_{2(i-1)}) \Delta t \quad (9)$$

and

$$G_{2i} = G_{01} / 2 + \sum_{j=1}^n c_1 a_3 T_F W_F S_F (1 - c_3 G_{2(i-1)}) \Delta t \quad (10)$$

The corresponding equations for *F. verticillioides* were

$$G_{2i} = G_E + \sum_{j=n}^N a_1 G_{2(i-1)} T_F W_F \Delta t \quad (11)$$

and

$$G_{2i} = G_{02} / 2 + \sum_{j=1}^n c_1 a_5 T_F W_F S_F \Delta t \quad (12)$$

The interference coefficients (c_2 and c_3 , $\%^{-1}$) in equations 9 and 10, when multiplied by the percent visual infection of *F. verticillioides*, slowed down the growth of *F. graminearum*. The initial values of growth were divided by two because only one-half the inoculums was used per species in the mixed treatment.

Fig. 1. Temperature function (lines) for *Fusarium graminearum* (Gr) and *F. verticillioides* (Ve) as used in the simulation model described in the text compared with growth data (symbols) from Reid et al. (15).

The next stage of the model was to relate growth of *Fusarium* to the concentration of toxin in an ear (T_X). To do this we used a second order polynomial:

$$T_X = b_1 G + b_2 G^2 \quad (13)$$

Experimental procedures. Two experiments were used in this study. Details of the first experiment (experiment A) were described in Reid et al. (15). A maize hybrid (DeKalb DK415) was planted in a 4×12 factorial experiment arranged in a split-plot design with three blocks in Ottawa, Canada, in 1992, 1993, and 1994. Four inoculation treatments were randomized among the main plot units so each treatment consisted of 12 single-row subplot units that corresponded to 12 harvest times. Inoculation treatments consisted of *F. graminearum*, *F. verticillioides*, the two species mixed together, and a control treatment of sterile water. After inoculation, plots were irrigated with 2 to 5 mm of water daily in the late afternoon for 28 days. Ears were collected at 12 harvest times: 2, 4, 6, 8, 10, 12, 14, 18, 22, 35, 49, and 63 days after inoculation. In 1992 and 1993, an additional five harvest times were added (1, 3, 5, 20, and 26 days after inoculation). Harvested ears were bulked within each row and hand shelled. Each sample was mixed thoroughly and a subsample was freeze-dried and ground to a fine powder for analyses of deoxynivalenol (DON) and fumonisin (FB₁).

Ear samples were rated for disease severity using a 7-class rating system where 1 = no visual infection, 2 = 1 to 3%, 3 = 4 to 10%, 4 = 11 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, and 7 = >75% of the kernels exhibiting visible disease symptoms (14). This scale represents a relatively quick and convenient method for quantifying disease severity but is logarithmic in nature. It can be converted to percent visual infection (% G), which is more closely related to the amount of *Fusarium* growth per ear. This conversion used a polynomial relating percent infection to the rating scale. Scale values of 1, 2, 3, and 4, etc., correspond to G values of 0, 1.5, 7, and 18%, etc., which were the middle value of percent visible infection of each class. Average hourly values of temperature and relative humidity and daily precipitation were recorded at the experimental site. Air temperatures were measured throughout the experiment, and silk channel temperatures were measured for about the first 20 days until the silk channel no longer existed due to cob growth. Silk channel temperatures were used in the model when available and then air temperatures were used. These silk channel temperatures were highly correlated with and quite similar to air temperatures (15). Substituting air temperatures for silk channel temperatures had virtually no impact on model calculations.

In a second experiment (experiment B), nine inoculation times (0, 1, 3, 6, 9, 12, 15, 20, and 30 days after silk emergence) were randomly assigned to three blocks of nine four-row plots of the maize hybrid used in experiment A. Three inoculation treatments (*F. graminearum*, *F. verticillioides*, and a sterile water control) were randomized among the four subplot rows. The inoculation procedures were the same as used in experiment A. Each subplot row consisted of 10 plants in a 3.8 m long row with 76 cm between rows. Irrigation procedures were repeated as in experiment A, and air temperatures and precipitation were recorded as in experiment A. At normal grain harvest in the middle of October, 10 ears (one from each plant) were hand-picked, husked, and evaluated for ear rot symptoms using the 7-class rating system described in experiment A.

In experiment A, we inoculated 5 days after first silk appearance and measured percent visual infection at specified time intervals during the growth period. In experiment B, we inoculated at specified times after first silk appearance and measured percent visual infection at harvest time.

Parameterization. There are a number of empirical coefficients in this model. The a coefficients (a_1 to a_5) had well-defined values when sums of squares between model calculations and ob-

servations from experiment A were plotted as functions of each coefficient and could be solved for by fitting the model to percent visual infection using a nonlinear least squares fitting algorithm (7) with numerical differentiation. In total, 138 observations over the 3 years of experiment A were used to solve for these coefficients. There are six coefficients because a_3 was species-dependent and therefore had two values. The c coefficients did not have well-defined minima, were interdependent on other coefficients, and could not be solved directly. These coefficients were determined in a sensitivity study in which values were varied systematically to obtain optimum fits (5). For example, c_1 was dependent to some extent on a_1 . Values of c_2 and c_3 determined the amounts of interference in the silks and kernels, respectively, but model output was insensitive to where the interference took place. Therefore, we assumed approximately equal interference in the silks and kernels. Initial values were also set by sensitivity studies since, to some extent, they interacted with the a_1 coefficient. Values of 0.3 and 0.6% visual infection were set for G_0 and G_E when species were inoculated separately, respectively. When the inoculum was mixed, values of G_0 were set at 0.15 for each species and the total amount of infection was calculated separately for each species and then added together.

We intended to use the model, as described above, to calculate values of infection for the conditions of experiment B as an independent test. That is, we started the model at each inoculation time in experiment B, ran it over the growing period, and compared the calculated percent visual infection with that measured at harvest. However, preliminary results showed the need for a silk function to account for the effect of silk age on growth. Reid et al. (13) showed that disease ratings increased to a maximum value and then decreased as silk inoculation times increased from time of first silk appearance. They made no attempt to separate age factors from environmental affects. In this study, we developed a silk function (S_F) to account for silk aging using the data from experiment B. S_F was developed as a function of modified growing degree days (MGDD) (18) accumulated from first silk appearance. S_F was set at a small negative number (d_1) at first silk appearance, increased to one at a specified number of MGDD (d_2), remained at one until another specified number of MGDD (d_3), where it declined with a slope d_4 (MGDD⁻¹) to a lower limit of d_5 . These coefficients (d_1 to d_5) were determined by comparing model calculations to the disease infection levels from experiment B through a sensitivity analysis with the exception of d_4 , which was solved directly by the nonlinear least squares method used previously but with the data from experiment B. The coefficients b_1 and b_2 from equation 13 were solved by least squares using data from Reid et al. (16).

RESULTS AND DISCUSSION

Infection from natural sources of inoculum, as determined from the control treatment, were negligible so that using percent visual infection for determining coefficients was valid. Values of all coefficients are shown in Table 1. Note, only the a coefficients and d_4 have standard errors that were calculated by the algorithm (7). The temperature function for each species (equation 2) was compared with growth rates of *Fusarium* spp. grown on infected silks on agar plates under controlled conditions from Reid et al. (15) (Fig. 1). We set values of T_{opt} and T_{max} for *F. graminearum* based on this data. However, the equivalent values for *F. verticillioides* were estimates assuming *F. verticillioides* grew faster at higher temperatures. A frequency distribution of temperatures for the first 40 days after inoculation (Fig. 2) indicated that a large percentage of the temperatures were below 30°C. As well, conditions in Ottawa, ON were not conducive to growth of *F. verticillioides* because growth rates of this fungus were relatively small each year. Therefore, the fitting procedure was not able to establish values for T_{opt} and T_{max} for *F. verticillioides*.

The data in Figure 1 suggests that T_{min} would be in the range of 22 to 24°C. However, as stated above, other studies (1,6) have indicated that growth of *F. verticillioides* can occur below 22°C. *F. graminearum* causes head blight in wheat (19), which happens during the relatively cool temperatures of June in Ontario. Therefore, a lower temperature limit (T_{min}) of 15°C seemed reasonable although research is continuing on temperature functions.

The a_2 coefficient was physically related to the boundary layer resistance of Rao et al. (12), which is related to the dimensions of the ear and the wind velocity in the vicinity of the ear. In this case, a_2 was also related to the water conductivity of the husks since part of the film of water was in the husks and between kernels and husks. Wind velocity at ear level was not measured in this experiment and is seldom available for field experiments. As well, there is a dearth of information as to how water moves back and forth from the husk surface to the kernels where growth of these fungi takes place. Thus, as a first approximation, a_2 was assumed to be a

Fig. 2. Relative frequency of temperatures for the first 40 days after inoculation for the conditions of experiment A.

simple constant that was solved for by the nonlinear algorithm and represented a value for an average-sized ear for average wind conditions. Even though the data was used to solve for this coefficient, a_2 will be related to the above factors that influence evaporation but not to the nature of how the water was applied. That is, we would still expect the value of a_2 in Table 1 to have the same value whether or not overhead irrigation was used to supplement rainfall unless irrigation wets the ears differently than rainfall. This is important for future use of this model for predicting occurrences of disease. The exact thickness of the film (equation 4) of water (1 mm) was not critical to the model. An increase or decrease of up to 50% (determined by sensitivity studies using the data from experiment A) could be tolerated because a_2 and a_3 would adjust to these changes.

The a_3 coefficients represented the response of *Fusarium* spp. to the wetness of the leaf, which was, in turn, determined by precipitation and the evaporative conditions of the atmosphere. The a_3 coefficient for *F. graminearum* was almost twice the value for *F. verticillioides* (Table 1). The temperature response functions for the temperatures measured in this experiment were very similar. Therefore, the large difference between the growth of the two species was determined mainly by a_3 .

TABLE 2. Time from silk inoculation to when the infection reaches the ear for *Fusarium graminearum* (Gr), *F. verticillioides* (Ve), and for mixed inoculation (Gr + Ve), as calculated from the model described in the text, and the initial values (G_E) of percent visual infection of the ear used in the model

Year	Species	Time (d)	G_E
1992	Gr	13.54	0.6
	Ve	15.63	0.6
	(Gr + Ve)	13.79	0.27 (Gr)
			0.33 (Ve)
1993	Gr	15.46	0.6
	Ve	21.54	0.6
	(Gr + Ve)	16.42	0.25 (Gr)
			0.35 (Ve)
1994	Gr	9.67	0.6
	Ve	12.75	0.6
	(Gr + Ve)	10.71	0.25 (Gr)
			0.35 (Ve)

Fig. 3. Five-day moving averages of cumulative precipitation deficits (millimeters) for 60 days after 1 June for the 3 years (1992 to 1994) of experiment A.

Initial starting values and growth in silks are very important and are interdependent on growth coefficients. We chose an initial value (G_0) of 0.3% to leave room for natural sources of disease invasion when we would assume a value between 0 and 0.3%. A G_E value of 0.6% was dependent on c_1 and a_1 . We determined all this by sensitivity studies using data from experiment A and determining reasonable values for the time it took G to increase from 0.3 to 0.6%. These times and component values of G_E are shown in Table 2. The component values represented the amounts of growth in the silks of each species for the mixed inoculum treatment. There was a possibility that amounts of inoculum reached saturated levels very quickly and that actual amounts of inoculum above the levels used in the mixed inoculum treatment would not affect results. If this was the case, this saturating effect can be programmed into the model but it would also be necessary to increase the values of the interference coefficients in the model.

The a_5 coefficient is intriguing. It had a value of 0.192 in 1993 (Table 1). It was assumed to be 1.0 in the other years. During 1993, growth rates down the silks were less than one-third that of the other years if all other factors were the same. Reid et al. (15) showed substantially less ergosterol in the silks for this year. We assumed that this was due to preinoculation weather conditions. Precipitation deficits during June and July indicated much drier conditions in 1993 compared with 1992 and 1994 (Fig. 3), which made the silks less susceptible to infection. We quantified this by calculating the precipitation deficit over the 40 days before the day of inoculation and relating it to this growth coefficient, which was 1.0 in 1992 and 1994 and 0.192 in 1993 (Fig. 4). The nonlinear nature of the function in Figure 4 was such that for most years this coefficient would equal 1.0 and not be a factor in the model, which was a reasonable assumption that has not been verified. Note that the function in Figure 4 was not used in the analyses in this paper and does not affect the results. Only the data points were used. The function was derived for future comparisons with other data sets and could change with more analyses.

The *Fusarium* spp. growth model compared quite well to the disease rating data and followed the yearly patterns of *Fusarium* spp. growth reasonably well both for isolated species and when they were inoculated together (Fig. 5). Note that the model follows a general sigmoid pattern that after inoculation there was a period with slow progress of fungal growth followed by exponential growth then by another slow growth. The overall R^2 was 0.91 with a root mean square error of standard error of estimate (SEE) of 2.42%, although this was not an independent test of the model. It is obvious from Figure 5 that in isolation, *F. graminearum* grew at a higher rate than *F. verticillioides*. However, when the two species were mixed together, values of percent visual infection for *Fusarium* spp. were much less than

Fig. 4. Water stress growth coefficients (a_5) for silks as a function of precipitation deficits (x axis) (millimeters) summed over 40 days prior to silking for 1992, 1993, 1994, and 1995.

when *F. graminearum* was inoculated alone. Part of this is because only half the amount of inoculum was used for each species to prepare the mixed inoculum treatment. This is accounted for in the model when we divided G_0 by two. However, we also had to assume some interference by *F. verticillioides* on the growth of *F. graminearum* to optimize the fits.

Simulated growth of *Fusarium* on the ears was exponential (equation 1), which enabled the model to calculate very large growth rates under favorable conditions. This growth was controlled in the latter parts of the growing period by lower temperatures because at these later stages of growth water availability was not as variable as temperatures (data not shown). Thus, the value of $T_{\min}$ was a critical factor in the model. We found that lowering $T_{\min}$ decreased the growth coefficient (a_1) and made growth more

linear with time. Values of $T_{\min}$ lower than 15°C resulted in relatively large SEEs with the data of Figure 5. Therefore, setting $T_{\min}$ to 15°C was a combination of using a value that worked well in the model but also seemed the most reasonable based on what we know of the *Fusarium* growth from the literature.

Fig. 5. Percent visual infection of maize ears as related to days from inoculation for *Fusarium graminearum* (Gr) by itself, *F. verticillioides* (Ve) by itself, and for the two species inoculated together (Gr + Ve) over 3 years. Lines indicate model calculations. Inoculation took place 5 days after silk emergence. Error bars represent the standard error of the means.

Fig. 6. Percent visual infection as related to time of inoculation (days from first silk appearance) for *Fusarium graminearum* (Gr) and *F. verticillioides* (Ve) with no silk function ($F_S = 1$). Symbols represent percent visual infection measured at harvest time. Lines are corresponding model calculations. Error bars represent the standard error of the means.

Fig. 7. The silk function (F_S) related to modified growth degree days accumulated from the time of first silk appearance as used for the conditions of experiment B.

The silk function was another key factor. When S_F was set to one throughout the growth periods, serious discrepancies appeared between model calculations and measurements of experiment B (Fig. 6), particularly 1 day after silk emergence. When we applied the silk function (Fig. 7), the model calculations were much closer to the observations of experiment B (Fig. 8). The R^2 improved from 0.58 to 0.89 and the SEE decreased from 20.8 to 5.9% although this was not an independent test of the model because the data of experiment B was used to develop the silk function. When model calculations were repeated for conditions of experiment A with the silk function in place, they did not change from the original model calculations for experiment A. However, a key assumption was that the silks aged from the outside in and that infection grew down the silk tubules faster than the aging process. This assumption was necessary because it took 13 to 22 days before there were significant levels of infection on the ears (Fig. 5; Table 2) and we assumed that this lag was due to the time taken for the *Fusarium* to grow down the silks. This may not be true. *Fusarium* may grow down silk more rapidly than what was assumed here but took time to switch from silk growth to growth on kernels. More research is required to resolve this aspect of *Fusarium* growth. What the model showed was that the condition of silks played a major role in disease infection. There appeared to be a very narrow window (4 to 6 days after silk emergence) for optimum conditions for infection. When silks first emerged they seemed to resist disease invasion but quickly (2 to 4 days) became susceptible. Maximum susceptibility ($S_F = 1$) only lasted for several more days when susceptibility decreased to a plateau level. This plateau level was put into the function so that there

Fig. 8. Percent visual infection as related to time of inoculation (days from first silk appearance) for *Fusarium graminearum* (Gr) and *F. verticillioides* (Ve) using the silk function depicted in Figure 7. Symbols represent percent visual infection measured at harvest time. Lines are corresponding model calculations. Error bars represent the standard error of the means.

would be some risk, however small, of infection through the silks during the latter stages of the growth period. This plateau level of 0.20 instead of zero improved the fit only marginally but it could be important for other data sets. Reid et al. (13) showed some disease in maize ears at harvest time when silks were inoculated 10 to 16 days from first silk appearance for two other hybrids.

Equation 13 related percent visual infection to toxin levels from Reid et al. (16) well ($R^2 = 0.87$) (Fig. 9). However, Reid et al. (15) showed that sometimes very high levels of DON can occur at relatively low levels of percent visual infection (Fig. 9). The fit in Figure 9 is conservative since two outlying points (which appeared to be spurious) were not plotted. Therefore, the relationship (equation 13) presented here may not be adequate. Further work is being conducted similar to Pitt (10) in which toxin formation is related to rate of growth rather than the amount of growth. The low levels of FB_1 corresponded to the small amount of *F. verticillioides* calculated by the model.

The model is developed according to most conventional thinking that *F. graminearum* grows better in wetter, cooler conditions than *F. verticillioides*. Under the weather conditions of Ontario, both *F. graminearum* and *F. verticillioides* are relatively common. However, in some years (usually wet years), *F. graminearum* is the predominant species that produces high levels of toxins. Results from this study suggest that *F. graminearum* has to outgrow the *F. verticillioides* sufficiently to overcome the interference. In hotter, drier conditions, *F. verticillioides* would prob-

Fig. 9. A, Toxin concentrations (y) are related to percent visual infection (x) using the data from Reid et al. (16). The solid line ($y = 3.66x + 0.025x^2$) (equation 13) is a least squares fit to the data with R^2 of 0.85. B, The data from Reid et al. (15) is compared with the same function. DON = deoxynivalenol; FB = fumonisin.

ably predominate since it seems to have the ability to interfere with the growth of *F. graminearum*.

The model development in this study was only a first step in a conceptual or mechanistic model of *Fusarium* growth. Because we used the data in experiment B to develop a silk function, we did not have a truly independent test of the model. However, model calculations for the conditions of experiment B when S_F equaled one (those made on the fourth day after first silk appearance) were in fact independent. This meant that only four measurements were available for testing and it is fair to ask how the model calculations would compare with other independent data. In this case, a truly independent data set will be difficult to obtain because some of the coefficients will change with hybrid. The silk function may be in this category. However, the silk function was not just a method of making the model fit the data. It quantified changes in the susceptibility of silks to invasion of *Fusarium* with time and temperature and has inherent usefulness on its own. The growth coefficients a_1 and a_3 will almost certainly change with susceptibility of hybrids but may be a useful way of quantifying susceptibility to *Fusarium* disease. Therefore, analyzing more data with this model may be aimed at determining how various coefficients change with hybrid more than at independently testing the model.

The environment of Ontario is not conducive to growth of *F. verticillioides*. It would be unreasonable to assume that we adequately tested a model for this species for all locations. In Ontario, *F. verticillioides* does not produce enough toxins to be a problem. *F. graminearum* is the species of most concern. The model, as presented, only dealt with infection through silks. Other means of the fungi for infecting the kernels, such as through insect wounds or bird damage, has not been considered here but are topics for ongoing research.

The ultimate use of this model is to calculate geographical locations and years when toxins are produced in large enough quantities of concern. However, we are several steps away from this. For one, the toxin submodel needs to be improved. As well, we have not included any aspects of natural sources of inoculum. However, the model does have uses in its present form. For example, the model quantified the importance of silk age to successful infection of maize ears by two species of *Fusarium* and demonstrated the importance of inoculating silks 4 to 6 days after silk emergence for testing silk resistance to infection of maize lines. If all lines do not silk within several days, they have to be inoculated on different days, which would result in having different environmental conditions for *Fusarium* infection and confounds inherent resistance with environmental conditions. This could be avoided if the percent visual infection measured on each line is expressed as a fraction of the percent visual infection calculated by this model.

In summary, a mathematical model was developed that successfully simulates growth of *F. graminearum* and *F. verticillioides* under separate and mixed silk inoculations. It is the first stage of a model to calculate natural levels of maize ear infection of *Fusarium* spp. for field conditions.

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LITERATURE CITED

1. Alberts, J. F., Gelderblom, W. C. A., Thiel, P. G., Marasa, W. F. O., Van Schalhwyk, D. J., and Behrend, Y. 1990. Effect of temperature and incubation period on production of Fumonisin B₁ by *Fusarium moniliforme*. Appl. Environ. Microbiol. 56:1729-1733.
2. Baier, W. 1971. Evaluation of latent evaporation estimates and their conversion to potential evaporation. Can. J. Plant Sci. 51:255-266.
3. Baier, W., and Robertson, G. W. 1965. Estimation of latent evaporation from simple weather observations. Can. J. Plant Sci. 45:276-284.
4. Campbell, C. L., and Madden, L. V. 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York.
5. Hunt, L. A., Pararajasingham, S., Jones, J. W., Hoogenboom, G., Imamura, D. T., and Oghoshi, R. M. 1993. GENCALC—Software to facilitate the use of crop models for analysing field experiments. Agron. J. 85:1090-1094.
6. Marin, S., Magan, N., Serra, J., Ramos, A. J., Canela, R., and Sanchis, V. 1999. Fumonisin B₁ production and growth of *Fusarium moniliforme* and *Fusarium proliferatum* on maize, wheat, and barley grain. J. Food Sci. 64:921-924.
7. Marquardt, D. W. 1963. An algorithm for least squares estimation of non-linear parameters. J. Soc. Ind. Appl. Math. 11:431-441.
8. Miller, J. D., Savard, M. E., Schaafsma, A. W., Siefert, K. A., and Reid, L. M. 1995. Mycotoxins production by *Fusarium moniliforme* and *Fusarium proliferatum* from Ontario and presence of fumonisins in the 1993 corn crop. Can. J. Plant Pathol. 17:233-239.
9. Moschini, R. C., and Fortugno, C. 1996. Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. Eur. J. Plant Pathol. 102:211-218.
10. Pitt, R. E. 1993. A descriptive model of mold growth and aflatoxin formation as affected by environmental conditions. J. Food Prod. 56:139-146.
11. Prelusky, D. B., Rotter, B. A., and Rotter, R. G. 1994. Toxicology of mycotoxins. Pages 359-403 in: Mycotoxins in Grain: Compounds Other than Aflatoxin. J. D. Miller and H. L. Trenholm, eds. Eagan Press, St. Paul, MN.
12. Rao, P. S., Gillespie, T. J., and Schaafsma, A. W. 1998. Estimating wetness duration on maize leaves from meteorological observations. Can. J. Soil Sci. 78:149-154.
13. Reid, L. M., Bolton, A. T., Hamilton, R. I., Woldemariam, T., and Mather, D. E. 1992. Effect of silk age on resistance of maize to *Fusarium graminearum*. Can. J. Plant Pathol. 14:293-298.
14. Reid, L. M., Hamilton, R. I., and Mather, D. E. 1996. Screening maize for resistance to Gibberella ear rot. Agric. Agri-Food Can. Tech. Bull. Publ. 196-5E.
15. Reid, L. M., Nicol, R. W., Ouellet, T., Savard, M., Miller, J. D., Young, J. C., Stewart, D. W., and Schaafsma, A. W. 1999. Interaction of *Fusarium graminearum* and *F. moniliforme* in maize ears: Disease progress, fungal biomass, and mycotoxin accumulation. Phytopathology 89:1028-1037.
16. Reid, L. M., Stewart, D. W., and Hamilton, R. I. 1996. A 4-year study of the association between Gibberella ear rot severity and deoxynivalenol concentration. J. Phytopathol. 144:431-436.
17. Shurtleff, M. C., ed. 1984. Compendium of Corn Diseases. The American Phytopathological Society, St. Paul, MN.
18. Stewart, D. W., Dwyer, L. M., and Carrigan, L. 1998. Phenological temperature response of maize. Agron. J. 90:73-79.
19. Sutton, J. C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Plant Pathol. 4:195-209.
20. Sutton, J. C., Baliko, W., and Liu, H. J. 1980. Fungal colonization and zearalenone accumulation in maize ears injured by birds. Can. J. Plant Sci. 60:453-461.
21. Vigier, B., Reid, L. M., Seifert, K. A., Stewart, D. W., and Hamilton, R. I. 1997. Distribution and prediction of *Fusarium* species associated with maize ear rot in Ontario. Can. J. Plant Pathol. 19:60-65.
22. Waggoner, P. E., Horsfall, J. G., and Lukens, R. J. 1972. EPIMAY, a simulator of southern corn leaf blight. Conn. Agric. Exp. Stn. Bull. 729.