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A Descriptive Model of Mold Growth and Aflatoxin Formation as Affected by Environmental Conditions

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ABSTRACT

A model is presented which integrates literature data on the effects of temperature, water activity, pH, and colony size on mold growth and aflatoxin formation. Mathematical forms for the rates of growth and toxin formation are based on assumptions about the biology of toxigenesis. The rate of toxin formation is assumed to be proportional to the rate of production of new cell mass, and the rate of toxin degradation is assumed to be proportional to the product of the concentrations of dead cell mass and aflatoxin; the latter assumption is an attempt to be consistent with the notion that toxin degradation is effected by enzymes released during mycelial lysis. Growth rate and toxin yield are represented by a maximum or reference value times a series of factors dependent on environmental conditions. Temperature and water activity have an interactive effect on growth and toxigenesis in the model. An Arrhenius-like function is postulated for the effects of temperature; shape parameters in the function are selected assuming that optimum temperature bears a fixed relationship to temperature limits for growth and toxigenesis, which vary with water activity. A linear function is postulated for the effect of water activity, with the lower limit dependent on temperature. Parabolic and Monod models are used to describe the effects of pH and colony size, respectively. Toxigenic parameters are estimated by comparing model simulations to the results of two published studies, with fair consistency in the two sets of parameters. In comparisons with other studies, the model did not correctly project the effects of spore load, but did correctly predict toxigenic behaviors relating to the effects of temperature and temperature cycling. The model provides a theoretical explanation for observed temporal shifts in the optimum temperature for toxigenesis, and for a hyperbolic relationship between heat units and time to toxigenesis with and without temperature cycling.

Production of aflatoxins by toxigenic strains of *Aspergillus flavus* or *Aspergillus parasiticus* is affected by a number of factors, including temperature, water activity (a_w), pH, substrate and oxygen concentrations, substrate type, and culture age. The environmental conditions resulting in aflatoxin formation have been well documented in studies in which one or more factors have been varied on an individual or combined basis (*13,17,21*). However the integration of these data into a single, comprehensive framework has not yet been done.

Mathematical modeling has been used to predict the extent of fungal growth and invasion of foods or feeds as dependent on environmental conditions (*4,11,16,20*). These models describe the rates of growth as governed by assumptions about mold biology and physical laws such as Fick's law of diffusion and thus are mechanistic or semi-mechanistic in nature. Unlike strictly empirical models which are limited to the conditions in which an experiment is performed, semi-mechanistic models can provide insight into the behavior of a biological system in more natural and uncontrolled environments and may serve to point out areas in which information is lacking.

Mathematical modeling of mycotoxin formation may be particularly difficult. Regulation of secondary metabolism is poorly understood (*14*), and the relationship between the rates of primary and secondary metabolism is not clear. For example, aflatoxin concentrations generally rise during the logarithmic and deceleration phases of growth (*23*), which suggests that the toxin is either a metabolite produced by growing cells or is converted biosynthetically from some other compound by growing cells. However, with ochratoxin A and citrinin produced by *Penicillium viridicatum* (*8*), high toxin levels were detected at 70-100 d after the beginning of the stationary phase. Thus, broad generalizations about the mechanisms of mycotoxin formation are difficult, and the formulation of mathematical models is therefore challenging.

Time course data for aflatoxin genesis usually exhibit a rise in concentration to a peak level, followed by a decay in concentration to near zero. The mechanisms of degradation or conjugation have not been conclusively demonstrated, which poses another difficulty for mathematical modeling. Ciegler et al. (*6*) noted that aflatoxin decay was coincident with a rapid rise in pH, a sudden rise in supernatant nitrogen, the initiation of mycelial lysis, and depletion of sucrose. Controlling pH or supplementing with sucrose did not affect toxin degradation, whereas fragmenting the mycelia increased degradation. Bacon et al. (*2*) suggested that degradation of F-2 toxin produced by *Gibberella zeae* was the result of action by enzymes released from cellular lysis, a notion ascribed to also by Montani et al. (*15*) for zearalenone produced by *Fusarium graminearum* in corn. Doyle and Marth (*9*) found that fragmentation of *A. parasiticus* mycelia increased toxin degradation rate but found also that the rate was higher

with greater initial toxin concentration and depended on mycelial age. Addition of *Paecilomyces* protoplasts isolated from grass silage caused more rapid degradation of patulin (*1*). The only consensus might be that mycelial lysis is concomitant with more rapid toxin degradation.

The objectives of this study were to (i) develop equations for the rates of production and degradation of aflatoxins as related to mold growth and environmental conditions; (ii) estimate characteristic toxigenic coefficients in the model from available literature data; and (iii) make qualitative comparisons between the model's behavior and data on the effects of temperature from studies not used in model development. Ultimately, a model of this type, when rigorously tested, may prove useful in predicting the risk of aflatoxin contamination in foods and feeds.

MATHEMATICAL MODEL

Basic model

The model consists of differential equations for the instantaneous rates of change of mold mass and aflatoxin concentration as dependent on environmental conditions. In line with the classic models of microbial growth (*20*), the rate of change of cell-mass concentration was proportional to net growth rate and current concentration.

$$\frac{dC_{mold}}{dt} = (\mu_g - \mu_m)C_{mold}, \quad [1]$$

where

C_{mold} = concentration of mold mass, g/g medium
t = time, h
μ_g = true growth rate, h^{-1}
μ_m = maintenance rate, h^{-1}.

Net growth rate $\mu_g - \mu_m$ varies with environmental conditions. Following Muck et al. (*16*), net growth rate was calculated as a maximum rate $(\mu_g - \mu_m)_{max}$ at optimum conditions, multiplied by a series of factors which reduced growth rate at suboptimum conditions:

$$\mu_g - \mu_m = (\mu_g - \mu_m)_{max} \cdot f_T \cdot f_{a_w} \cdot f_{pH} \cdot f_{mold} \quad [2]$$

where

f_T = relative growth rate as dependent on temperature
f_{a_w} = relative growth rate as dependent on a_w
f_{pH} = relative growth rate as dependent on pH
f_{mold} = relative growth rate as dependent on mold mass.

The last factor imposes a growth limit due to substrate depletion or diffusion limitations.

The form of the equation for aflatoxin production reflects an assumption about mechanism. If toxins are produced independently of growth, then the rate of production may be proportional to mold mass (C_{mold}). If toxins are produced simultaneously with growth, then the rate of production may be proportional to growth rate. It was decided to make the rate of toxin formation proportional to both growth rate and cell mass, whence

$$R_p = Y_p \cdot \mu_g \cdot C_{mold} \quad [3]$$

where

R_p = rate of aflatoxin production, g toxin/(g medium -h)
Y_p = toxin yield coefficient, g toxin/g cell mass.

The assumption in equation [3] is that each increment in new cell mass is accompanied by an increment in new toxin mass. This assumption would be inappropriate in cases where toxin levels increase after growth ceases.

Environmental conditions affect toxin formation differently from growth (*17*). To accommodate this fact, the toxin yield coefficient was made a separate function of temperature, a_w, and pH, using again a series of factors:

$$Y_p = Y_p^{(max)} \cdot g_T \cdot g_{a_w} \cdot g_{pH} \quad [4]$$

where

$Y_p^{(max)}$ = maximum yield in conditions optimum for toxigenesis
g_T = relative yield as dependent on temperature
g_{a_w} = relative yield as dependent on a_w
g_{pH} = relative yield as dependent on pH.

A mechanistic assumption is also required for describing toxin degradation. Some alternatives are hereby considered. If degradation is nonenzymic, then the rate may be proportional simply to toxin concentration. If toxins are conjugated by growing molds, then degradation rate may be proportional to live cell mass, or to live cell mass times growth rate. If toxins are degraded by enzymes released by mycelial lysis, then degradation rate may be proportional to dead cell mass. This last case would seem to be the most likely scenario (*6,9*); however, to incorporate the effect of toxin concentration on degradation rate as observed by Doyle and Marth (*9*), the rate was assumed proportional to concentrations of both toxin and dead cell mass:

$$R_d = \mu_d \cdot C_{dead} \cdot C_{toxin} \quad [5]$$

where

R_d = degradation rate, g toxin/(g medium -h)
μ_d = degradation coefficient, h^{-1}
C_{dead} = dead cell-mass concentration, g/g medium
C_{toxin} = toxin concentration, g/g medium.

In connection with the fact that toxin stability decreases with increasing temperature (*14*), the degradation coefficient was assumed to depend on temperature:

$$\mu_d = \mu_d^{(ref)} \cdot h_T \quad [6]$$

where

$\mu_d^{(ref)}$ = degradation coefficient at a reference temperature of 20°C, h^{-1}
h_T = factor dependent on temperature.

h_T was assumed to follow an Arrhenius relationship that doubled between 20 and 30°C.

Cell death was assumed synonymous with maintenance (*18,20*), hence:

$$\frac{dC_{dead}}{dt} = \mu_m \cdot C_{mold} \quad [7]$$

Finally, the net rate of change of toxin concentration was the difference between the rates of production and degradation:

$$\frac{dC_{toxin}}{dt} = R_p - R_d \quad [8]$$

Effects of environmental conditions

Schindler et al. (*21*) provided data for the effects of temperature on growth and aflatoxin production by *A. flavus* at a fixed a_w. Response to temperature was skewed, and the optimum temperature for toxigenesis was less than that for growth. Cuero et al. (*7*) and Northolt and Bullerman (*17*) showed that temperature and a_w have an interactive effect on the conditions limiting growth and toxigenesis. Thus, the functions f_T and g_T for temperature must depend on a_w, and the functions f_{a_w} and g_{a_w} must depend on temperature.

An Arrhenius-like temperature function was developed with flexible parameters that would accommodate different temperature limits for growth or toxigenesis as dependent on a_w:

$$f_T, g_T = A \cdot \exp\left\{-\left(\frac{\alpha^2}{T - T^{(min)}} + \frac{\beta^2}{T^{(max)} - T}\right)\right\} \quad [9]$$

where

$T^{(min)}$ = minimum temperature for growth or toxigenesis, °C
$T^{(max)}$ = maximum temperature for growth or toxigenesis, °C
T = temperature, °C
α,β = shape parameters
A = scaling parameter.

The optimum temperature for growth or toxigenesis (obtained by setting the derivative of [9] to zero) was given by

$$T^{(opt)} = \frac{\alpha T^{(max)} + \beta T^{(min)}}{\alpha + \beta} \quad [10]$$

The parameter A was selected to yield f_T, $g_T = 1$ at $T = T^{(opt)}$.

The shape of the temperature function is controlled by α and β. When α = β, the optimum temperature falls halfway between $T^{(min)}$ and $T^{(max)}$, and equation [9] is symmetric about $T^{(opt)}$. A difference between α and β creates skewness that offsets the optimum temperature to one side or the other. As α and β increase, keeping their ratio constant, the curve progresses from an inverted U to an increasingly bell-shaped configuration.

It was decided to select α and β on the basis of the temperature response of Schindler et al. (*21*) at fixed a_w, then to assume α and β remained constant as temperature limits varied with a_w. Thus, optimum temperature was assumed to bear a fixed relationship to the minimum and maximum temperatures as a_w varied. Fig. 1 shows the data of Schindler et al. (*21*) for relative growth and aflatoxin formation and the functions f_T and g_T with α and β fitted by nonlinear regression. For growth, the optimum temperature was such that α = 1.5β. For toxigenesis, α = β. Table 1 gives the estimated parameters and goodness of fit.

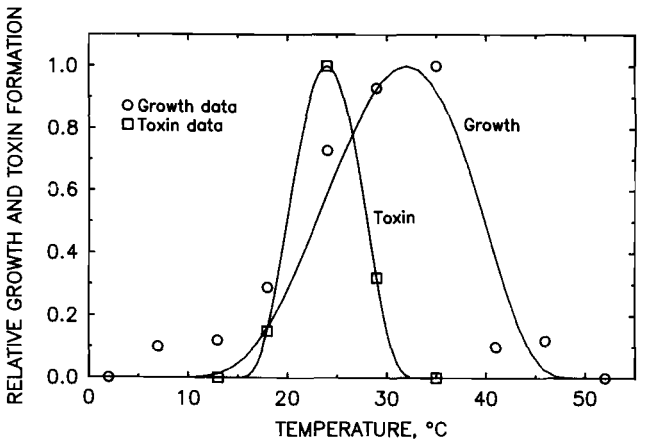

Figure 1. *Model functions for the effects of temperature on growth and toxin formation fitted to the data of Schindler et al. (21).*

TABLE 1. *Parameters for temperature response of growth and aflatoxin formation estimated from data of Schindler et al. (21).*

	α	β	Goodness of fit (r^2)
Growth	9.58	6.39	0.900
Toxigenesis	4.93	4.93	>0.999

Temperature limits for growth and aflatoxin formation as dependent on a_w were obtained from Northolt and Bullerman (*17*) by regressing a parabolic function for a_w versus T. For growth, the function was (r^2 = 0.990)

$$a_w = 1.172 - 0.02557T + 0.0004368T^2 \quad [11]$$

At a given T, values of a_w larger than that given by equation [11] permitted growth. If a_w is fixed, the minimum and maximum temperatures for growth could be calculated by reversing equation [11] using the quadratic formula:

$$T_g^{(max,min)} = 29.270 \pm \sqrt{856.71 - 2289.4(1.172 - a_w)} \quad [12]$$

where subscript 'g' refers to growth, and $T_g^{(max)}$ is associated with the '+' sign. A similar regression of the aflatoxin graph from Northolt and Bullerman (*17*) gave (r^2 = 0.970)

$$a_w = 1.197 - 0.02468T + 0.0004126T^2. \quad [13]$$

For a given T, values of a_w above that produced by equation [13] permitted toxigenesis. For a given a_w, the temperature limits were

$$T_p^{(max,min)} = 29.908 \pm \sqrt{894.48 - 2423.7(1.197 - a_w)} \quad [14]$$

where subscript 'p' refers to aflatoxin production.

Based on data from Hocking and Pitt (*12*) for *Penicillium oxalicum* and *Penicillium expansum,* Muck et al. (*16*) used a linear relationship to describe the effect of a_w on growth. For the present study, growth rate and toxigenesis both were assumed to vary linearly with a_w between 1.0 and a minimum a_w dependent on temperature:

$$f_{a_w}, g_{a_w} = \frac{a_w - a_w^{(min)}}{1 - a_w^{(min)}}. \quad [15]$$

Actual optimum a_w for toxigenesis is less than 1.0 on some substrates (*5*) but was not modeled in this study. The minimum a_w for growth or toxigenesis at a given temperature was calculated from equation [11] or [13], respectively.

For lack of information, the effect of pH was assumed to be independent of temperature and a_w. Data from Buchanan and Ayres (*3*) for growth and aflatoxin production by *A. parasiticus* at varying pH were used to develop f_{pH} and g_{pH}. Optimum pH for growth was observed to be about 5.0, which was lower than that of 6.0 for toxigenesis; decreasing pH had a stronger effect on toxigenesis than on growth. Parabolic functions, symmetric about the optimum pH, were regressed against the data (*3*). For growth (r^2 = 0.825):

$$f_{pH} = 1 - 0.0644\ (pH - 5)^2. \quad [16]$$

For aflatoxin formation (r^2 = 0.962):

$$g_{pH} = 1 - 0.1048\ (pH - 6)^2. \quad [17]$$

Growth rate declines as mold mass approaches a peak concentration, presumably because of substrate depletion. Normally, a Monod model would be used to represent the effect of substrate concentration on growth (*20*); however, in some studies the initial concentration of available substrate is not known, so concentration of substrate could not be calculated through time. To circumvent this problem, a Monod model was still used to limit growth, but with a maximum mold concentration determining when growth ceased:

$$f_{mold} = \frac{C_{mold}^{(max)} - C_{mold}}{K_m + C_{mold}^{(max)} - C_{mold}} \left\{\frac{K_m + C_{mold}^{(max)}}{C_{mold}^{(max)}}\right\}, \quad 0 \le C_{mold} \le C_{mold}^{(max)} \quad [18]$$

where

K_m = constant, g/g medium.

The term within brackets { } ensures that f_{mold} = 1 when C_{mold} = 0. The magnitude of K_m determines how soon growth decreases as C_{mold} approaches $C_{mold}^{(max)}$. When $K_m >> C_{mold}^{(max)}$, f_{mold} assumes a linear profile between 0 and $C_{mold}^{(max)}$. Values of K_m will be selected later.

Maximum net growth rate $(\mu_g - \mu_m)_{max}$ was initially taken as 0.363 h^{-1}, based on the literature survey of Muck et al. (*16*). Separate calculations of μ_g and μ_m were required for equations [3] and [7], respectively. It was assumed that temperature and mold concentration would have the same effect on true growth and maintenance (*16*), which is consistent with studies showing mold mass to remain constant after reaching a peak (*23*). At optimum growth conditions, the ratio of maintenance rate and true growth rate (μ_m/μ_g) was estimated by Muck et al. (*16*) to be approximately 0.114, based also on a literature survey. These assumptions led to the equations (*16*):

$$\mu_g = \mu_g^{(max)} \cdot f_T \cdot f_{mold} \quad [19]$$

$$\mu_m = \mu_g^{(max)} \cdot f_T \cdot f_{mold} (1 - 0.886 \cdot f_{pH} \cdot f_{aw}) \quad [20]$$

where

$\mu_g^{(max)} = 0.40\ h^{-1}$ (16).

Equation [20] would be inappropriate if maintenance rate remained constant when substrate was depleted (20), in which case f_{mold} would be absent from equation [20].

Equations [1] through [20] form the mathematical model and were solved numerically by computer using Newton's method with a time step of 1 h.

MODEL CALCULATIONS AND COMPARISONS WITH EXPERIMENTS

Unknown parameters remaining in the model include the toxin yield coefficient $Y_p^{(max)}$, the degradation coefficient $\mu_d^{(ref)}$, and the Monod constant K_m. These were estimated by fitting the model results approximately to literature data in which time courses of growth and aflatoxin concentration at known temperature, a_w, and pH were reported. (When not given, pH was assumed to be 6.0 and a_w to be 0.99.) Subsequently, the model's behavior was qualitatively compared with studies not used in obtaining toxigenic parameters.

Shih and Marth (23)

Time course data were reported for mycelial dry mass, aflatoxin concentration, lipid concentration, pH, and residual glucose concentration with *A. parasiticus* grown at 15, 25, 35, or 45°C on a synthetic growth medium. Growth was relatively rapid at 25 and 35°C and became limited at about 2 g dry weight (DW) per 100 ml medium, at which point glucose was depleted. At 35 and 45°C, essentially no toxin was formed. At 25°C, toxin levels increased in rough proportion to DW up to about 30 ppm at 5 d, then declined by about 30% over the next 2 d. At 15°C, toxin levels rose slowly to about 15 ppm at 7 d.

The goal was first to imitate the growth curve, then to estimate the toxigenic parameters. Thus, $C_{mold}^{(max)}$ was set at 0.02 g/g medium, and K_m was set at 1 g/g medium which produced a linear f_{mold}. Maximum net growth rate $(\mu_g - \mu_m)_{max}$ was also reduced by half to 0.2 h^{-1} after it was determined that growth in the model was too rapid. Because a_w was not reported, the temperature limits functions, equations [12] and [14], could not be employed. From the Shih and Marth data (*23*), it was evident that the minimum temperature for growth was below 15°C, the maximum temperature for growth was above 45°C, and the optimum temperature was about 35°C. These were not consistent with the Northolt and Bullerman (*17*) functions at any a_w, so instead the temperature limits of Schindler et al. (*21*) were used, namely 2-52°C for growth, 13-35°C for toxigenesis.

Figure 2 shows the projected growth curves at 25 and 35°C and the Shih and Marth data drawn from their Fig. 3. To estimate the toxigenic parameters, the toxin yield coefficient $Y_p^{(max)}$ was first varied to obtain an approximately correct peak toxin concentration, then the degradation coefficient $\mu_d^{(ref)}$ was varied to match the concentration at 7 d. This exercise resulted in

$Y_p^{(max)} = 0.0015$ g/g

$\mu_d^{(ref)} = 0.5\ h^{-1}$

As shown in Fig. 2, the model generated toxin levels that peaked just at the end of the deceleration phase of growth (day 3.5), whereas the data peaked at about day 5. At 35 and 45°C, toxins were neither generated by the model nor observed in the data. However, mold growth in the model was inhibited at 15 and 45°C, indicating the model was still too restrictive of growth at the temperature extremes.

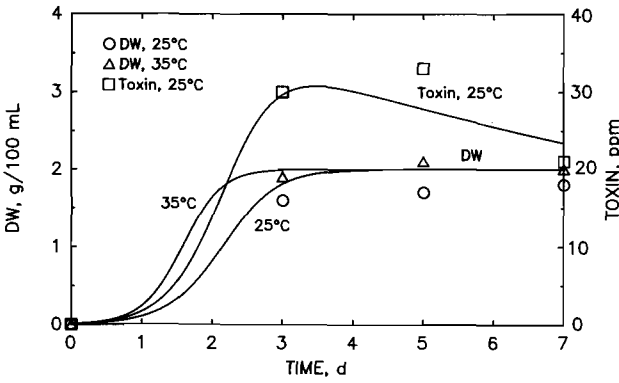

Figure 2. *Simulation of mycelial dry weight (DW) and aflatoxin concentration at two temperatures in comparison with the DW and toxin data of Shih and Marth (23).*

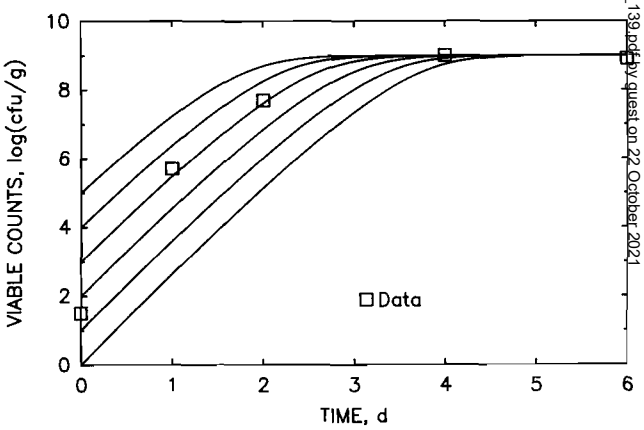

Figure 3. *Simulation of viable colony counts for various initial counts at 28°C and data for one level of inital spore load from Karunaratne and Bullerman (13).*

Karunaratne and Bullerman (13)

Time course data were presented for viable colony count and aflatoxin B_1 and G_1 concentrations for *A. parasiticus* grown at 28 and 35°C on rice . Eight initial spore counts, 10° to 10^7/g medium, were used. At both temperatures, colony counts increased to about 10^9 CFU/g and remained constant thereafter. Smaller initial spore counts

required longer to reach the maximum count. At 28°C, highest toxin levels were achieved with the 10^3/g spore load. The maximum toxin concentration was 360 ppm of aflatoxin B_1 on day 5, after which toxin concentration declined to about 120 ppm on day 10. Levels of aflatoxin G_1 were smaller by a factor of 3-4. At 35°C, growth was faster, but toxin levels were much lower and were still increasing at day 10.

To simulate this study, the original $\mu_g^{(max)}$ of 0.40 h^{-1} and the temperature limits of Schindler et al. (*21*) were used (a_w was not reported). Initial counts were taken to be those on day 1 to allow time for spore germination. Since the model was based on cell-mass concentration, a conversion factor was required to relate colony count to cell mass. Pitt et al. (*19*) used an approximate value of 10^{-9} g/CFU. However, this value was evidently too large for the present study in which peak counts were 10^9 CFU/g. Thus, a cell mass of 10^{-10} g/CFU was employed, although this figure should probably change with colony age. A Monod constant, K_m = 1 log (CFU/g), was assumed.

Figure 3 shows the simulated growth curve for various initial colony counts at 28°C. These approximate the actual data fairly well, although the time to reach 10^9 CFU/g starting at 10° CFU/g was too short. Toxigenic parameters were estimated as before from the data for 10^3 spores per g, with a satisfactory fit obtained with

$Y_p^{(max)}$ = 0.002 g/g

$\mu_d^{(ref)}$ = 0.25 h^{-1}.

Figure 4 shows the simulated toxin concentrations and data for the 10^3/g spore load. The shape of the curve bore some resemblance to the actual data, having a small initial slope and a concave-upward shape during degradation. However, the effects of changing initial colony count in the model were not corroborated by the data. The model-generated curves reached the same peak with each initial count, with the time to reach the peak shifting downward with larger initial counts. The depression of peak toxin concentrations at higher or lower spore loads in the data would suggest there is some biological effect of spore germination on toxigenesis that is not realized in the model.

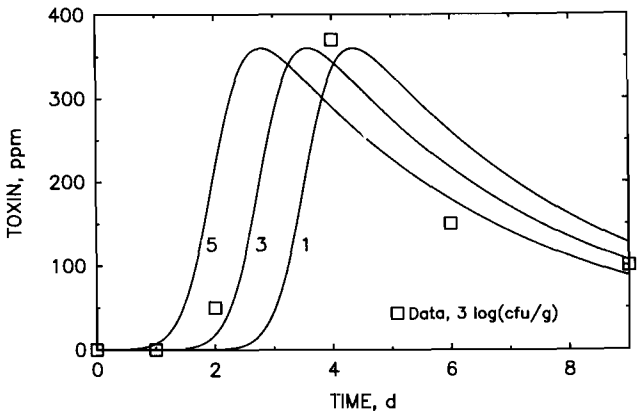

Figure 4. *Simulation of aflatoxin concentration for various initial colony counts [log(CFU/g)] and comparison with data for one level of initial count from Karunaratne and Bullerman (13) at 28°C.*

The estimated toxigenic parameters for this study were quite consistent with those for the Shih and Marth (*23*) study, within an order of magnitude. Buchanan and Ayres (*3*) reported total aflatoxins produced per unit mass of mycelium by *A. parasiticus* over 7 d at 28°C and varying pH; their yields varied from 0.001 to 0.008 g/g cell mass, which are again consistent with the $Y_p^{(max)}$ estimated here within an order of magnitude. Epstein et al. (*10*) reported mycelial and aflatoxin B_1 and G_1 concentrations at various temperatures and fixed times for *A. flavus* grown in liquid medium. The ratio of these values at room temperature was about 0.001 g toxin per g mycelial mass, which is again consistent with the other values.

Schroeder and Hein (22)

Time course data were presented for total aflatoxin production by *A. flavus* on three substrates at several temperatures. Toxin concentrations increased rapidly between days 1 and 6, then stayed roughly constant up to day 10. Two key results were obtained. First, between 20 and 30°C, the time at which toxin concentrations reached a measurable level decreased with increasing temperature. Second, the temperature at which toxin concentrations were maximum decreased with time.

Quantitative comparisons with these data were not possible because mold growth was not reported. Parameters set for the Shih and Marth (*23*) study were used. Fig. 5 shows the simulated growth and toxin curves at temperatures from 20 to 30°C. As temperature increased from 20 to 27°C, both cell mass and aflatoxin accumulated more rapidly. Similar to the Schroeder and Hein data (*22*), the time to reach an (arbitrary) toxin concentration of 5 ppm decreased from 4 to 1.3 d as temperature increased from 20

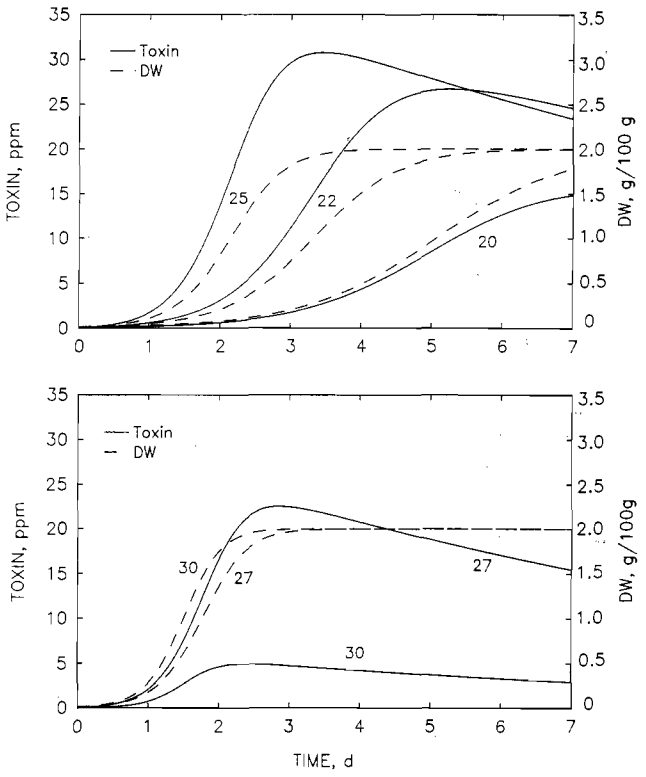

Figure 5. *Simulation of the effects of temperature on mold dry weight (DW) and aflatoxin concentration. Top: temperatures of 20, 22, and 25°C; bottom: temperatures of 27 and 30°C.*

to 30°C. At 30°C cell mass accumulated more rapidly, but aflatoxin accumulated more slowly and reached a smaller peak. These results would be expected intuitively.

The model results took on a less intuitive character when they were plotted in the same way as Fig. 2 of Schroeder and Hein. Fig. 6 shows aflatoxin concentration on a logarithmic scale plotted against temperature for various fixed times. Qualitatively, this figure bears a strong resemblance to the Schroeder and Hein figure, most notably that as time progressed, the temperature for maximum toxin concentration decreased. Although quantitative differences existed between the maximum toxin levels and the optimum-temperature shifts, the model would seem to provide theoretical support for the trends observed by Schroeder and Hein.

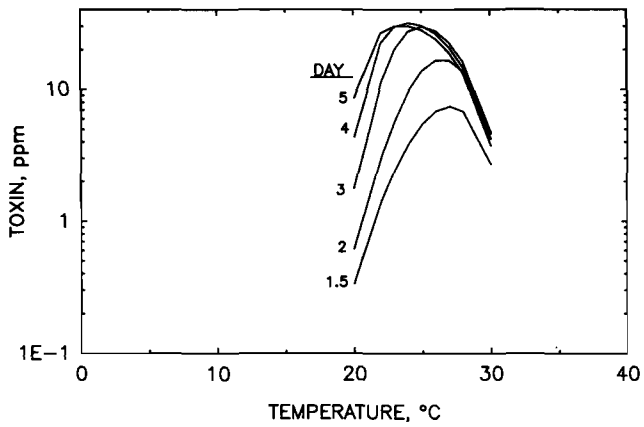

Figure 6. *Simulated results of aflatoxin concentration versus temperature at various times. The downward shift of optimum temperature with increased time is consistent with Schroeder and Hein (22).*

Stutz and Krumperman (24)

The effects of temperature cycling on time to sporulation and aflatoxin formation by *A. parasiticus* were considered in this study. A basis of comparison between constant and cycled temperature regimes was formed in terms of heat input, defined to be the area under the temperature-time curve divided by total time (deg-h/d). As heat input increased, time to produce aflatoxin decreased. However, for the same heat input, the time was shorter when temperature was cycled than when it was held constant, except near the minimum and maximum heat inputs (270 and 770 deg-h/d, respectively) where cycling had little effect.

To compare the model with this study, the data of Stutz and Krumperman were slightly reinterpreted. First, the heat input as defined by Stutz and Krumperman was observed to be identically the same as average temperature over the incubation period:

$$\bar{T} = \frac{1}{t_{total}} \int_0^{t_{total}} T(t)dt \qquad [21]$$

where

$\bar{T}$ = average temperature

t_{total} = total time, h.

To restore the units of deg C, the heat input in deg-h/d was divided by 24. Thus, the minimum and maximum heat inputs corresponded to $\bar{T}$ of about 11 and 32°C, respectively.

Second, the constant-temperature data in Stutz and Krumperman suggested a hyperbolic relationship between time to toxin formation and $\bar{T}$:

$$t_p = \frac{b_1}{\bar{T} - b_2} \qquad [22]$$

where

t_p = time to produce aflatoxin, d

b_1, b_2 = constants.

Note that $t_p \to \infty$ as $\bar{T} \to b_2$, which shows that b_2 represents the minimum temperature for aflatoxin formation. Estimation of b_1 and b_2 from their data was carried out by rearranging equation [22]:

$$t_p \cdot \bar{T} = b_1 + b_2 \cdot t_p \qquad [23]$$

in which $t_p \cdot \bar{T}$ represents heat units, in deg-d, required for toxin formation. Equation [23] is an appropriate representation of the constant-temperature data of Stutz and Krumperman if the measured $t_p \cdot \bar{T}$ plot linearly against t_p (Fig. 7, top). Linear regression of these data produced (r^2 = 0.999):

b_1 = 1.38 deg-d

b_2 = 11.4°C.

Data for cycled temperatures from Stutz and Krumperman (*24*) are also shown in Fig. 7 and plot as a line with smaller slope on the axes shown. To account for cycling, equation [22] was modified by adding a third term to the denominator:

$$t_p = \frac{b_1}{\bar{T} - b_2 + b_3 \Delta T} \qquad [24]$$

where

ΔT = temperature amplitude (half the difference between higher and lower temperature levels), °C

b_3 = constant.

Note that for constant temperature, ΔT = 0 and equation [24] reverts to [22]. Since b_1 and b_2 were already known, it remained to estimate b_3 for the case of temperature cycling. Rearrangement of equation [24] yields

$$t_p\bar{T} - b_2 t_p - b_1 = -b_3 t_p \Delta T. \qquad [25]$$

A linear regression of the data was formed between the function of t_p and $\bar{T}$ on the left-hand side of the equation, and the quantity $t_p\Delta T$ on the right-hand side, with zero intercept. This resulted in (r^2 = 0.853):

b_3 = 0.106.

Model parameters were set using the values from Shih and Marth (*23*), and temperature cycling at 12-h intervals was simulated starting at the higher temperature level. Fig. 8 shows the simulated toxin concentrations at average temperatures of 20, 25, and 30°C. Each graph in Fig. 8 shows curves for temperature amplitudes of 0, 1, 3, and 6°C. At $\bar{T}$ = 20°C (top), cycling the temperature caused toxin levels to increase rapidly when temperature was at the higher level and to stay constant or decay when tempera-

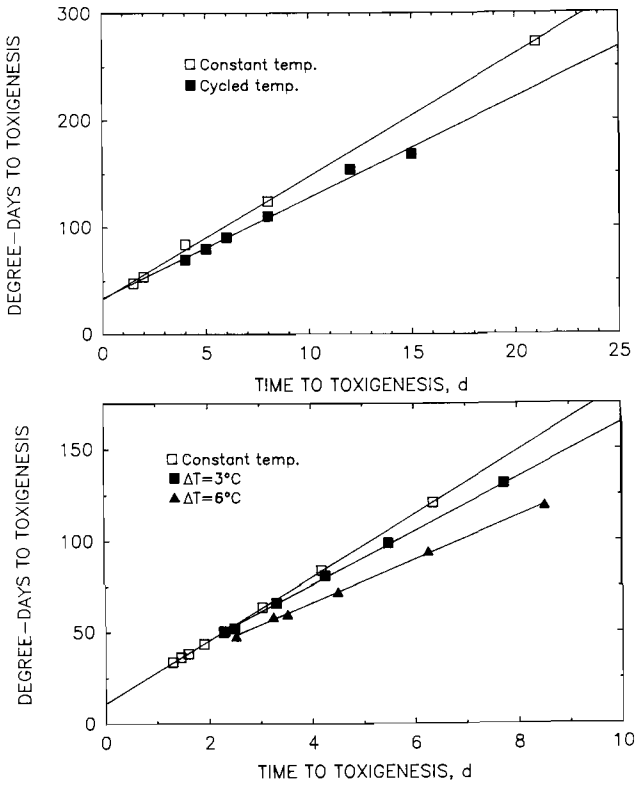

Figure 7. *Relationship between heat units (deg-d) to aflatoxin genesis and time to aflatoxin genesis, with and without temperature cycling. Top: data from Stutz and Krumperman (24); bottom: simulation results with constant temperature and with temperature amplitudes of 3 or 6 °C.*

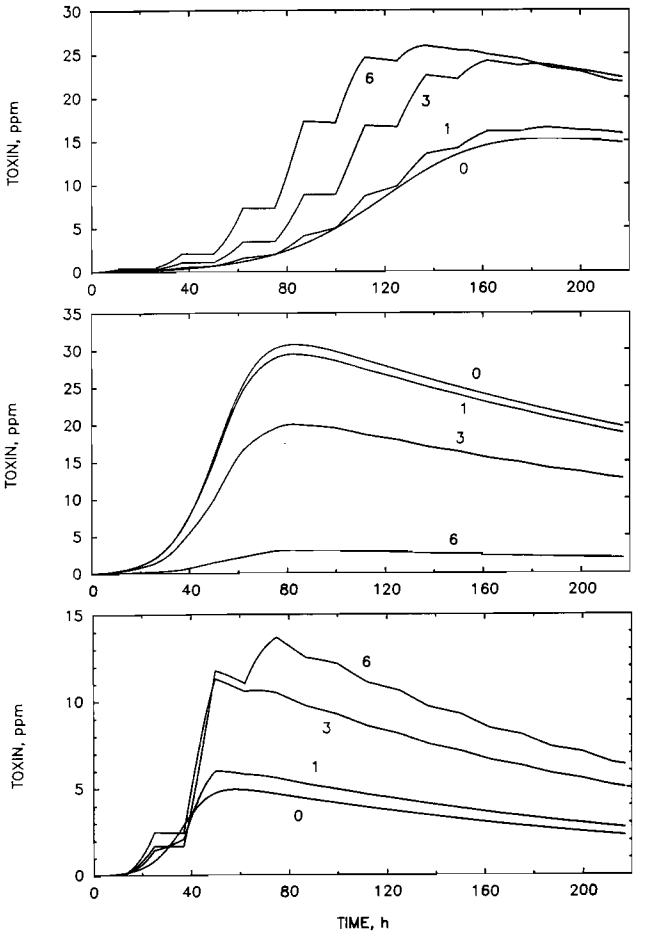

Figure 8. *Simulated aflatoxin concentration versus time with temperature amplitudes of 0, 1, 3, or 6°C. Top: average temperature of 20°C; middle: average temperature of 25°C; bottom: average temperature of 30°C.*

ture was at the lower level. As amplitude ΔT increased, toxin levels increased more rapidly. Evidently, the lack of accumulation at the lower temperature level was more than offset by the faster accumulation at the higher level.

At $\overline{T}$ = 25°C (Fig. 8, middle), the opposite pattern emerged. Here, increasing amplitude caused aflatoxin to accumulate more slowly and reach a lower peak. Different rates of accumulation at the high and low temperature levels were not as evident as for $\overline{T}$ = 20°C. Because the optimum temperature for toxigenesis was close to 25°C, cycling the temperature above and below this value reduced the rate of toxigenesis at both the high and low temperature levels.

At $\overline{T}$ = 30°C (Fig. 8, bottom), the pattern was again reversed, with larger amplitudes resulting in greater toxin accumulations. Because 30°C was supraoptimum for toxigenesis in the model, toxin accumulation was more rapid at the lower temperature level, enough so to offset the decay in toxin concentration at the higher temperature level.

Figure 7, bottom, shows the model results with constant and cycled temperatures plotted in the same way as for the data of Stutz and Krumperman (Fig. 7, top). The vertical axis shows heat units $\overline{T} \cdot t_p$, and the horizontal axis shows t_p, the time to reach an (arbitrary) toxin concentration of 5 ppm. Different points are shown for different temperature amplitudes ΔT = 0, 3, or 6°C. As in Fig. 7, top, a straight line was formed for constant temperature and for each temperature amplitude. Also as before, the model generated lines with smaller slopes when temperature was cycled. The points shown were generated only for $\overline{T}$ between the minimum and optimum temperatures for toxigenesis, which was also the case for the Stutz and Krumperman data (*24*).

Qualitatively, the model provides a theoretical explanation of the data of Stutz and Krumperman. No adjustment was made to the model's equations for growth and toxigenesis to create an effect of temperature cycling on mold behavior. Thus, the equations developed from data at constant temperature resulted in the correct behavior at cycled temperatures. This would suggest that temperature cycling did not have a special biological effect on *A. parasiticus* in the study of Stutz and Krumperman beyond that anticipated from the effect of high and low temperatures observed when temperature was constant.

CONCLUSIONS

Parameters for a simulation model of mold growth and aflatoxin formation were estimated from two experimental studies in which environmental conditions and time courses of growth and toxigenesis were reported. A toxin yield coefficient of 0.0015 - 0.0020 g toxin per g new cell mass and a toxin degradation coefficient of 0.25 - 0.50 h^{-1} were determined, with fairly good consistency in the parameters estimated from the two studies.

In a qualitative comparison with data from Schroeder and Hein (*22*), the model correctly projected a decrease in optimum temperature for aflatoxin formation with increasing time. However, compared to data from Karunaratne and Bullerman (*13*), the model did not accurately represent the effects of spore load on maximum toxin concentrations, which suggests the existence of a biological factor not currently taken into account in the model.

Comparison with a study by Stutz and Krumperman (*24*) on the effects of temperature cycling showed good qualitative agreement. The model correctly projected a linear relationship between heat units (deg-d) required for aflatoxin formation and time to aflatoxin formation, with the slope of the line decreasing as the amplitude of the temperature cycle is increased.

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