

Development of a Model To Predict Growth of *Clostridium perfringens* in Cooked Beef during Cooling

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MS 04-207: Received 11 May 2004/Accepted 30 June 2004

ABSTRACT

The objective of this work was to develop a new model to predict the growth of *Clostridium perfringens* in cooked meat during cooling. All data were collected under changing temperature conditions. Individual growth curves were fit using DMFit. Germination outgrowth and lag (GOL) time was modeled versus temperature at the end of GOL using conservative assumptions. Each growth curve was used to estimate a series of exponential growth rates at a series of temperatures. The square-root model was used to describe the relationship between the square root of the average exponential growth rate and effective temperature. Predictions from the new model were in close agreement with the data used to create the model. When predictions from the model were compared with new observations, fail-dangerous predictions were made a majority of the time. When GOL time was predicted exactly, many fail-dangerous predictions shifted toward the fail-safe direction. Two important facts regarding *C. perfringens* should impact future modeling research with this organism and may have broader food safety policy implications: (i) the normal variability in the response of the organism from replicate to replicate may be quite large (1 log CFU) and may exceed the current U.S. Food Safety Inspection Service performance standard, and (ii) the accuracy of the GOL time model has a profound influence upon the overall prediction, with small differences in GOL time prediction (~1 h) having a very large effect on the predicted final concentration of *C. perfringens*.

Clostridium perfringens is a known foodborne pathogen commonly associated with meat and poultry products. It has been implicated as the cause of foodborne illness associated with ingestion of contaminated roast beef, turkey, meat-containing Mexican foods, and other meat dishes (6). Fifty-seven confirmed *C. perfringens* outbreaks, involving 2,772 cases, occurred between 1993 and 1997, and more than one third (35%) of these cases were attributed to meat products or mixed dishes containing meat (7). Angulo et al. (1) reported that the most common cause of *C. perfringens* outbreaks (40.9%) was improper cooling of food products. Although the number of samples testing positive for this organism is low, e.g., spores were detected in only 2 of 197 raw ground-meat samples at 3 to 100 spores per g (16), *C. perfringens* continues to be a major concern to the food industry.

Foodborne illness results when spores survive the cooking process and germinate to produce vegetative cells, which then multiply in cooked foods when the rate and duration of cooling are too slow (4). *C. perfringens* generation time has been reported to be as rapid as 7.4 min in autoclaved ground beef under optimal temperature conditions (37 to 45°C), and growth has been reported at temperatures as low as 6°C (25). Although the ingestion of spores has not been shown to be harmful, vegetative cells in the consumed food may pass through the stomach to the intestinal tract, where they sporulate and release enterotoxin (11).

The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) guidelines for the cooling of meat products state that uncured meat and poultry product should be cooled from 130°F (54.4°C) to 80°F (26.7°C) in no more than 1.5 h, and from 80°F (26.7°C) to 40°F (4.4°C) within 5 h. When meat processors are unwilling or unable to meet this cooling schedule, they must be able to prove that an alternative cooling regimen will result in less than a 1 log CFU increase in *C. perfringens* (23). Similar recommendations also exist in the Food and Drug Administration model food code for retail and food-service establishments (9).

Computer models are becoming more useful and thus more popular for helping to solve food microbiology problems (19), and there are models that can describe microbial behavior under changing temperature conditions (3, 5, 27). Previous studies in our laboratory (21) have shown that one published and widely known *C. perfringens* model (15) used in the USDA Pathogen Modeling Program (24) consistently underpredicted net growth. The objective of this study was to develop a mathematical model that accurately predicted the growth of *C. perfringens* under different cooling rates in cooked beef.

MATERIALS AND METHODS

Test organisms and spore production. Three strains of *C. perfringens*, NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10240 (Hobbs serotype 13), were obtained from Dr. V. K. Juneja (USDA Agricultural Research Service, Eastern Regional Research Center, Philadelphia, Pa.). These same strains have been widely used in *C. perfringens* research and mod-

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el development and are applicable for research in beef (15). The strains were maintained and prepared according to procedures described by Juneja et al. (12). Appropriate volumes of a spore cocktail (approximately 10^8 CFU/ml) were inoculated into 908 g of ground beef (25% fat) that was obtained at a retail store. Initial spore concentration can influence germination and growth of *C. botulinum* (26) and other spore-forming bacteria (8, 18), so initial concentrations of approximately 10 and 10^3 spores per g were used for each cooling time to determine the effect of initial spore concentration.

The ground beef and culture were blended for 10 min on high speed (level 6) in the sterilized bowl of a mixer (model no. K45SS, KitchenAid, Inc., Greenville, Ohio). This mixing process ensured even dispersion of spores, which was verified by withdrawing numerous samples from a variety of positions within the mixing bowl. Statistical analysis indicated no significant differences between locations within each batch of inoculated meat. The mixing was halted once every minute to break apart large meat pieces and to remove residual meat from the sides of the bowl and mixing paddle. Three grams of inoculated ground beef was aseptically weighed into a sterile stomacher filter bag (SFB-510, Spiral Biotech, Boston, Mass.). Bags were vacuum sealed using a retail vacuum-packaging system (Food Saver Vac 300, Tilia, Inc., San Francisco, Calif.) and frozen until used (no longer than 4 weeks). Initial spore concentrations were evaluated over the 4 weeks of frozen storage to ensure that concentrations did not decrease during storage. Pouches were thawed at 4.4°C overnight, and then spores were heat activated at 75°C for 20 min in a circulating water bath. Heat shocking of samples simulates the cooking step in the process. This procedure is identical to that used to develop the original *C. perfringens* model (15) we originally sought to validate (21).

Temperature variation studies. All experiments were carried out in a programmable water bath (model no. 1028P, Fisher Scientific, Pittsburg, Pa.). Cooling of food products follows an exponential rate (14), so the water bath was programmed to mimic an exponential cooling rate during the dynamic temperature studies. The driving force for this cooling was assumed to be 0°C . Cooling was monitored using a thermocouple inserted into uninoculated ground beef samples. Samples were removed at appropriate intervals throughout each cooling process. Samples were diluted 1:10 in 0.1% sterile peptone water and pummeled in a stomacher (Lab Blender 400, model BA6021, Cooke Laboratory Products, Alexandria, Va.) for 2 min. Aliquots (1 ml) were pour plated with 12 to 15 ml of tryptose-sulfite-cycloserine agar with egg yolk emulsion and incubated at 37°C in Gas Pak containers (Baltimore Biological Laboratory, Cockeysville, Md.) to ensure an anaerobic environment.

Single cooling rate experiments investigated exponential cooling from 54.4 to 4.4°C over 6.5, 9, 12, 15, 18, 21, or 24 h. This range of cooling times includes the maximum USDA recommended time (6.5 h) and times that resulted in significant growth of *C. perfringens* in previous studies (18 h) (14). Dual cooling rate experiments were performed to simulate cooling deviations that might occur in the processed meat industry. FSIS guidelines state that a meat product should not remain between 54.4 and 26.7°C for longer than 1.5 h or between 26.7 and 4.4°C for longer than 5 h (23). The time periods associated with the proper 6.5-h cooling schedules were altered from 1.5 and 5 h to 3 and 3.5 h. Cooling times of 7 and 9 h were also investigated with variations proportional to the temperature ranges of the FSIS cooling guidelines. Predictions from the Juneja 1999 model were

used to guide the selection of experimental conditions to ensure that a range of growth responses would be observed.

Data analysis. Data were compiled and plotted using Sigma Plot version 8.0 (SPSS, Inc., Chicago, Ill.). Log-transformed data were fit using linear and nonlinear regression techniques using SAS statistical software (SAS Institute, Inc., Cary, N.C.). Individual growth curves were fit to the Baranyi and Roberts model (2) using the DMFit 1.0 Microsoft Excel add-in program (Institute of Food Research, Norwich Research Park, Norwich, UK; www.ifr.bbsrc.ac.uk). The DMFit 1.0 output includes predicted counts, calculated germination, outgrowth, and lag (GOL) times, and exponential growth rates (EGRs) for each fitted curve. Only curves where growth of greater than 1 log CFU/g was observed with R^2 values greater than 0.90 were used for development of the model.

GOL time model development. Data collected during the single rate cooling studies were used to create the predictive model. The log GOL time was plotted against temperature at the end of the GOL time (the temperature the water bath had reached at the end of the GOL time while cooling from 54.4 to 4.4°C). The four data points bounding the most conservative edge or the bottom of the data set were selected for modeling. Linear regression was used to model the relationship between log GOL time and temperature.

EGR model development. Because the temperature was constantly changing over the course of every experiment, each growth curve can be viewed as a series of observations taking place at a series of different temperatures. Every observation of *C. perfringens* concentration has an associated temperature. Every pair of observations can be thought to have occurred at an effective temperature, which is the temperature of the sample at the effective time. Effective time is the average of the two times when the *C. perfringens* concentration was measured. Figure 1 includes an example data set and the calculation of an effective temperature and an effective time. Thus, each growth curve (which takes place under changing temperature conditions) can be used to estimate a series of growth curves, each characterized by a concentration change at an effective temperature. An effective temperature value was calculated for each consecutive pair of data points from the DMfit output within the exponential growth phase of the curve. EGR values were determined from the slope of a linear regression line between the two subsequent points. The square-root model (20) was used to describe the relationship between the square root of the average EGR and effective temperature.

Evaluation of the predictive model. Data collected during the dual cooling rate cycles were used to evaluate the model for growth of *C. perfringens* during cooling. The time-temperature combinations used for dual cooling rate experiments were entered into an Excel spreadsheet. The designated models and parameters were also entered into the computer spreadsheet, and GOL time and EGR were estimated for each short interpolated time interval in the cooling process. The fraction of the GOL time expended during each time interval was calculated. Once the spores expended 100% of their GOL, the EGR model was used to predict growth.

RESULTS

There was no significant influence of inoculum size on net growth during any experiment. All six replicates at each cooling condition (three experiments with an initial inoculum concentration of 10 log CFU/g and three experiments

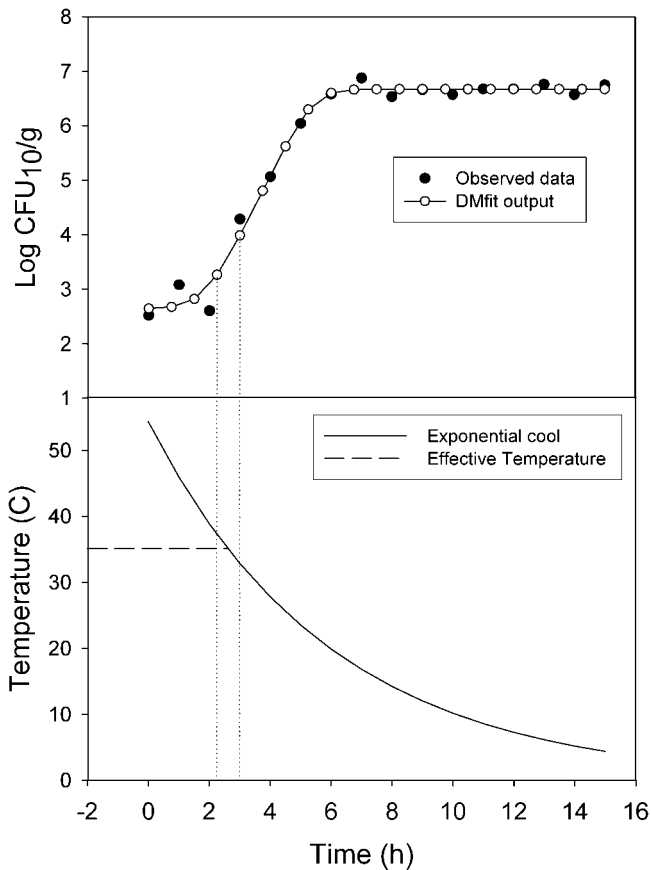


FIGURE 1. Observed and fitted *C. perfringens* growth data from one replicate cooled from 54.4 to 4.4°C over 15 h. Top: Method used to calculate effective time (t_{eff}): change in temperature (°C) over time (h) during a 15-h cooling period from 54.4 to 4.4°C. Bottom: Method to calculate effective temperature (T_{eff}).

with an initial inoculum level of 10^3 log CFU/g) were pooled prior to additional data analysis.

GOL time model. An examination of the relationship between GOL time and temperature at the end of GOL time showed a distinct trend among different cooling times (Table 1). As expected, the shortest cooling times (12 h) resulted in the lowest temperatures at the end of GOL time, whereas the longest cooling times (24 h) resulted in the highest temperatures at the end of GOL time. Intermediate cooling times (15, 18, and 21 h) resulted in temperatures at the end of GOL time between the 12- and 24-h temperatures. A more detailed description of the model development process can be found elsewhere (22). The lower boundary of the data set was used to model the relationship between temperature and GOL time to provide a fail-safe model prediction. Linear regression was used to model the relationship between log GOL time and temperature with the resulting relationship:

$$\log \text{GOL} = -0.0331 \times T + 1.8 \quad (1)$$

where GOL time is measured in hours and T is the temperature (°C).

EGR model. The square root EGRs versus effective temperature were plotted for each segment of each growth

TABLE 1. Effect of cooling rate on germination, outgrowth, and lag (GOL) time of *C. perfringens* and corresponding temperature at end of GOL time

Time (h) to cool from 54.4 to 4.4°C	End of observed GOL time	
	Time (h)	Temperature (°C)
12	2.3	42.6
	2.1	43.7
15	1.6	47.6
	2.1	45.7
	1.8	46.6
	2.3	44.9
18	1.5	48.8
21	1.8	48.8
	1.6	49.4
	2.6	46.7
	1.7	49.2
	1.7	49.3
	2.5	46.8
	2.1	48.7
24	1.5	50.4
	1.6	49.9
	1.2	51.0
	2.2	48.5
	2.1	48.7

curve from each experiment for all five cooling times (Fig. 2). An increase in EGR was observed with an increase in effective temperature until approximately 37°C. At effective temperatures above 37°C, EGR decreased. Little change in EGR was seen above effective temperatures of 46°C. This trend (increasing EGR up to 37°C, decreasing EGR to 46°C, followed by a plateau) was generally apparent for all five cooling times. The trend was more pronounced during the longer cooling times, during which EGRs increased at a more dramatic rate, peaked, and then decreased at an increased rate when compared with the shorter cooling times. The shorter cooling times (12, 15, and 18 h) growth took place at effective temperatures lower than 20°C, but no

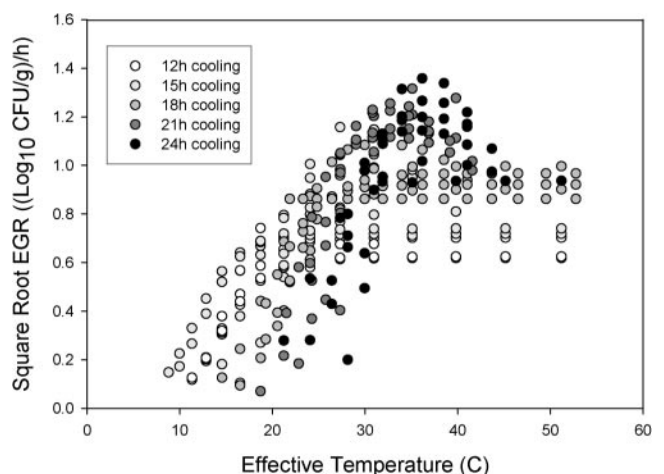


FIGURE 2. The square root of exponential growth rate (EGR) values (log CFU per gram per hour) of *C. perfringens* versus effective temperature (°C).

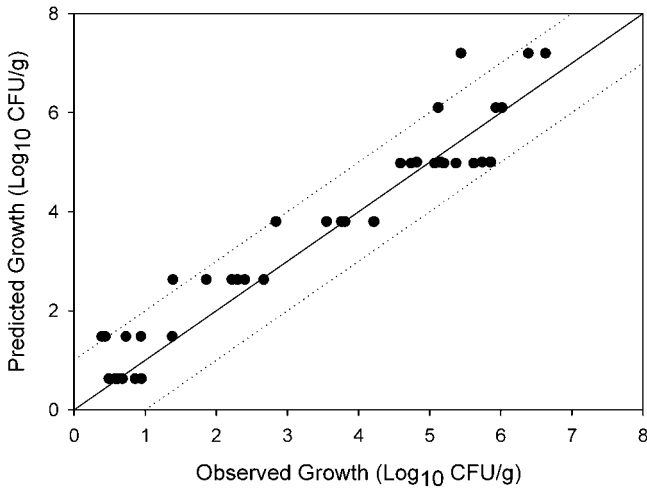


FIGURE 3. Predicted versus observed growth values for *C. perfringens* during cooling at one rate. Dotted lines represent ± 1 log CFU/g.

growth was seen at temperatures lower than 20°C during longer cooling times (21 and 24 h).

The square-root model (20) was used to describe the relationship between the square root of the average EGR and effective temperature and can be expressed by equation 2:

$$\sqrt{\text{EGR}} = 0.075 \times (T - T_{\min})[1 - e^{0.023(T-T_{\max})}] \quad (2)$$

where T is the effective temperature (in °C) and T_{\min} and T_{\max} are regression constants determined to be 7.71 and 63.27°C, respectively.

Evaluation of the model. Predictions from the new model were in close agreement with observations used to create the model (single rate cooling data) (Fig. 3). In many cases the variability in the observed data collected under identical conditions exceeded 1 log CFU/g, but most of the observed data points were within ± 1 log CFU of the predictions (dotted lines in Fig. 3). In the few cases where data points fell outside this range, the predictions were fail-safe (more growth was predicted than was observed). In one case where fail-dangerous predictions were noted, less than a 1 log CFU/g increase in *C. perfringens* was observed and predicted. In the other fail-dangerous situations, both the predictions and observed data were well over the 1 log CFU/g performance standard. Use of our model in these situations would not result in a violation of the USDA FSIS standard.

Predictions from the new model were also compared with observations from independent studies using dual cooling rate schedules (Fig. 4). The variability of the observed data collected under identical conditions exceeded 1 log CFU/g, as with the single rate data above. Also, a majority of the data points fell below the diagonal line, indicating that fail-dangerous predictions were made. In some cases, the predictions differed from the observed data in the fail-dangerous direction by more than 1 log CFU/g. There were a number of observations where greater than 1 log CFU/g of growth was observed but less than 1 log CFU/g was predicted (enclosed by dotted lines), which has serious

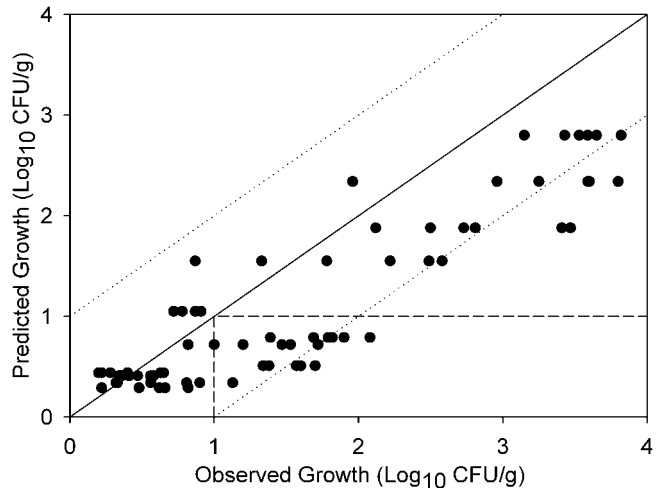


FIGURE 4. Predicted versus observed growth values for *C. perfringens* during cooling at two rates. Dotted lines represent ± 1 log CFU/g. Points enclosed by dashed lines represent conditions where less than 1 log CFU/g growth was predicted but more than 1 log CFU/g growth was observed.

implications for the suitability of our model in determining whether a particular cooling profile has met the USDA FSIS performance standard.

Further analysis was performed to determine the cause of underpredictions seen in Figure 4. Figure 5 is a plot of predicted versus observed growth values using the EGR model but assuming the GOL time could be predicted exactly. Although the variability of the observed data collected under identical conditions still exceeds 1 log CFU/g, use of the actual GOL time with the EGR model shifts many of the predictions toward the fail-safe direction. Far fewer points fall on the fail-dangerous side of the solid line, only four observations are fail dangerous by more than 1 log CFU/g, and a single observation (inside the dotted line)

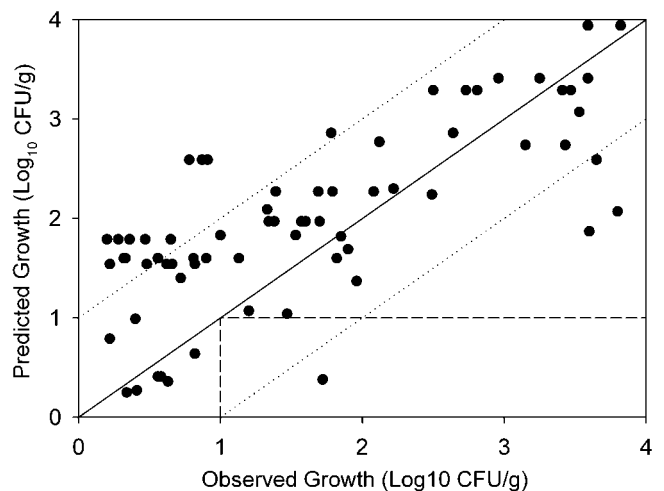


FIGURE 5. Predicted versus observed growth values for *C. perfringens* during cooling at two rates when GOL time is predicted exactly. Dotted lines represent ± 1 log CFU/g. Points enclosed by dashed lines represent conditions where less than 1 log CFU/g growth was predicted but more than 1 log CFU/g growth was observed.

has more than a 1 log CFU/g increase when less than a 1 log CFU/g increase was predicted.

DISCUSSION

Although data from growth at constant temperatures have been used successfully to predict growth of organisms during changing temperature situations (3, 5, 27), recent studies in our laboratory (21) have indicated that a published *C. perfringens* model (15) used in the USDA Pathogen Modeling Program consistently underpredicted net growth. Results from other models to predict the growth of *C. perfringens* in uncured beef under changing temperatures either are not relevant (e.g., Juneja et al. (13) studied growth in cooked cured beef) or have dealt with only primary models for growth and do not include secondary models for predicting growth as a function of temperature (10, 17).

In the study presented here, we used data collected under changing temperature conditions to develop and evaluate a new model for the growth of *C. perfringens* in beef during cooling. Evaluation of the model suggested that it may be used to accurately predict growth of *C. perfringens* at a single cooling rate and that these results deviate from the time-temperature guidelines given by the USDA FSIS, although additional validation data are needed because all the single cooling rate data presented here were used to develop the model.

When applied to dual cooling rate scenarios the predictions developed from single rate data were not as accurate and often underpredicted growth. Our analysis revealed that selection of an appropriate GOL time model is essential for accurate predictions under changing temperatures. Although the GOL time model developed here is not ideal, it does offer significantly improved predictions over the model developed by Juneja et al. (15).

Because the GOL time determines when a spore will begin exponential growth and because the *C. perfringens* EGR can be quite rapid (depending upon temperature) and the temperature at which a cell begins exponential growth is also dependent on GOL time, even slight differences in predicted GOL time can have important consequences, even when the EGR model is quite accurate. When GOL times from the single rate and dual rate experiments were analyzed systematically (analysis not shown), spore crop age had a significant impact on GOL time under changing temperature conditions: older spores (8 to 9 months) had longer GOL times, and younger spores (3 to 4 months) had shorter GOL times. Further analysis of these findings is currently underway.

This study was undertaken in an attempt to develop better models for predicting *C. perfringens* growth under changing temperature conditions. These models would be useful for determining whether a particular batch of product that was not cooled according to USDA FSIS guidelines would still meet the performance standard, which states that less than a 1 log CFU/g increase in *C. perfringens* should take place during the cooling process.

The results of this study have allowed us to make important progress toward this goal but have also uncovered

several important facts regarding *C. perfringens* that suggest that the performance standard itself may be unworkable. The variability in the response of the organism itself under well-controlled laboratory conditions may be greater than the entire performance standard. There are poorly understood methodological factors (e.g., spore age) that may have a great impact on the growth observed under identical conditions. The accuracy of the GOL time model has a profound influence upon the overall prediction, and the accuracy that is attainable given the normal variability seen in biological systems may not be suitable for making predictions regarding a 1 log CFU/g increase in the bacterial population. All these observations point to the need to re-evaluate the current performance standard.

A copy of the spreadsheet containing the model is available at <http://foodsci.rutgers.edu/schaffner/files.htm>.

ACKNOWLEDGMENT

This work was supported by a Cooperative State Research, Education, and Extension grant from the USDA.

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