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Effects of spontaneous heating on fiber composition, fiber digestibility, and in situ disappearance kinetics of neutral detergent fiber for alfalfa-orchardgrass hays¹

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ABSTRACT

During 2006 and 2007, forages from 3 individual hay harvests were utilized to assess the effects of spontaneous heating on concentrations of fiber components, 48-h neutral detergent fiber (NDF) digestibility (NDFD), and in situ disappearance kinetics of NDF for large-round bales of mixed alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.). Over the 3 harvests, 96 large-round bales were made at preset bale diameters of 0.9, 1.2, or 1.5 m, and at moisture concentrations ranging from 9.3 to 46.6%. Internal bale temperatures were monitored daily during an outdoor storage period, reaching maxima (MAX) of 77.2°C and 1,997 heating degree days >30°C (HDD) for one specific combination of bale moisture, bale diameter, and harvest. Concentrations of all fiber components (NDF, acid detergent fiber, hemicellulose, cellulose, and lignin) increased in response to spontaneous heating during storage. Changes in concentrations of NDF during storage (poststorage – prestorage; Δ NDF) were regressed on HDD using a nonlinear regression model ($R^2 = 0.848$) that became asymptotic after Δ NDF increased by 8.6 percentage units. Although the specific regression model varied, changes (poststorage – prestorage) in concentrations of acid detergent fiber, cellulose, and lignin also increased in nonlinear relationships with HDD that exhibited relatively high coefficients of determination ($R^2 = 0.710$ to 0.885). Fiber digestibility, as determined by NDFD, was largely unaffected by heating characteristics except within bales incurring the most extreme levels of HDD or MAX. In situ assessment of ruminal NDF disappearance kinetics indicated that disappearance rate (K_d) declined by about 40% within the range of heating incurred over these hay

harvests. The change in K_d during storage (ΔK_d) was related closely to both HDD and MAX by nonlinear models exhibiting high R^2 statistics (0.907 and 0.883, respectively). However, there was no regression relationship between changes (poststorage – prestorage) in effective ruminal disappearance of NDF and spontaneous heating, regardless of which heating measure was used as the independent variable. The close regression relationship between ΔK_d and measures of spontaneous heating indicates clearly that ruminal NDF disappearance was altered negatively by some direct or indirect aspect of spontaneous heating. However, it was equally apparent that these effects were offset by an expanding pool of dry matter recovered as potentially degradable NDF.

Key words: disappearance kinetics, fiber composition, hay, neutral detergent fiber

INTRODUCTION

Currently, producers in the north-central United States who harvest dairy-quality (>151 relative feed value) alfalfa in small rectangular bales receive approximately \$200/t for this hay product. Unfortunately, the harvest of alfalfa and other hays can be complicated by poor drying conditions or the threat of unexpected rainfall events, each of which potentially places this valuable cash crop at risk. As a result, it is common for producers to bale their hay crop before adequate desiccation has occurred or subject their wilting hay to rain damage.

Hay that is baled too wet commonly undergoes spontaneous heating, in which plant sugars are respired into CO₂, water, and heat by microorganisms associated with the hay (Rotz and Muck, 1994). Although the threshold moisture for acceptable storage for small (~45-kg) rectangular bales is approximately 20% (Collins et al., 1987), thresholds for larger hay packages are assumed generally to be lower, but they vary with the size and density of bales, as well as other factors. In addition to oxidation of nonstructural carbohydrates (Coblenz et al., 1997a), the phenomenon of spontaneous heating is

Received November 21, 2008.

Accepted February 16, 2009.

¹Mention of trade names or commercial products in this article is solely for the purpose of providing specific information, and does not imply either recommendation or endorsement by the US Department of Agriculture.

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associated with mold growth (Roberts, 1995), deleterious changes in forage nutritive value (Collins et al., 1987; Coblenz et al., 1996, 2000; Turner et al., 2002), alteration of ruminal disappearance kinetics of N, fiber, and DM (Coblenz et al., 1997b; McBeth et al., 2003; Turner et al., 2004), and less desirable measures of in vivo disappearance of DM (Montgomery et al., 1986; McBeth et al., 2001; Turner et al., 2004).

In previous studies (Coblenz et al., 1996, 2000), concentrations of NDF, ADF, cellulose, and lignin have been related to various indicators of spontaneous heating in positive linear relationships that are often characterized by relatively high coefficients of determination (r^2). Positive relationships between concentrations of fiber components and measures of spontaneous heating are assumed to occur primarily by indirect mechanisms; the respiratory activity of microorganisms has a concentrating effect on fiber constituents by oxidizing non-fiber compounds (Rotz and Muck, 1994), rather than actually creating additional forage fiber. In addition, it is frequently assumed that the digestibility of forage fiber is reduced by spontaneous heating, but support for this premise based on in vivo or other assessments is limited. Several studies have reported relationships between in vivo NDF digestibility and heating that were mildly negative (McBeth et al., 2001), inconclusive (Turner et al., 2004), or even positive when steam-treated (100 to 110°C for 0.75 h) alfalfa-hay-based diets were offered to lactating dairy cows (Broderick et al., 1993). Using in situ procedures, rates of ruminal NDF disappearance (K_d) for heated bermudagrass [*Cynodon dactylon* (Pers.) L.] hays were slowed by spontaneous heating during storage, resulting in reduced estimates of effective NDF degradability (McBeth et al., 2003), but effects on dairy-quality legumes remain unclear. An additional unknown factor to consider when evaluating past work is that previous studies have largely been confined to small (~45-kg) rectangular bale packages that were easy to replicate statistically, but typically exhibit limited heating characteristics compared with the large-round or large-square packages that are increasingly popular with hay producers. Within this context, Montgomery et al. (1986) reported reduced in vivo digestibility coefficients for cellulose and ADF within wethers consuming heated alfalfa-orchardgrass hays packaged in large-round bales.

In a companion report (Coblenz and Hoffman, 2009), we have evaluated spontaneous heating, DM recovery, whole-plant ash, in vitro true digestibility (IVTD), and ruminal in situ disappearance kinetics of DM for large-round bales of alfalfa-orchardgrass hay from 3 harvests. In that report, concentrations of IVTD and effective ruminal disappearance of DM were reduced in close association with spontaneous heating. It remains

unclear whether this occurred strictly as the result of respiratory losses of highly digestible forms of DM during storage, or whether these reductions were related to changes in the composition and subsequent degradability of fiber, or a combination of both mechanisms. The objective of this report was to relate changes in concentrations of fiber components, 48-h NDF digestibility (NDFD), and characteristics of ruminal in situ NDF disappearance to measures of spontaneous heating for the large-round bales of alfalfa-orchardgrass hay described within the companion report (Coblenz and Hoffman, 2009) using linear and nonlinear regression techniques.

MATERIALS AND METHODS

Field Procedures

This project comprised 3 separate hay harvests conducted on the same 8.2-ha field site over a 2-yr period (2006 and 2007). All details relating to the establishment of forages, soil fertility, harvest management, storage procedures, temperature measurements, and pre- and poststorage sampling procedures have been described within the companion report (Coblenz and Hoffman, 2009), and will be summarized here only briefly.

Pretrial Considerations. To meet the objectives of this project, several a priori decisions concerning methodology were made that require explanation. From an experimental design standpoint, one overall goal of the project was to establish a wide continuum of heating characteristics that contained temperature responses from both very dry and very wet hays. Unfortunately, it was not possible to meet this goal adequately within a single study using the 8.2-ha field site that was available; therefore, it was necessary to combine data over 3 separate harvests. We intentionally chose to confine prestorage bale moistures within each harvest to relatively narrow ranges, hereafter designated as low- (LM), intermediate- (IM), and high-moisture (HM). This approach provided logistical advantages, including increased flexibility with respect to labor schedules, while also providing a mechanism to accumulate sufficient forage to meet our research needs.

Source of Hays. Briefly, the 8.2-ha field site comprised a mixture of 'Phabulous II' alfalfa and 'Extend' orchardgrass that were established on April 14, 2004, near Stratford, Wisconsin. The LM and HM harvests utilized the second and third cuttings, respectively, harvested during 2006, whereas the IM harvest utilized forage from the second cutting of 2007. Respective dry weight percentages of alfalfa in LM, IM, and HM harvests were 91, 76, and 68%, whereas orchardgrass comprised 9, 22, and 31% of each sward. During each

harvest, forage was mowed and conditioned (model 8830, J. I. Case Co., Racine, WI), adjacent rows gathered with a bi-fold rake, and then hay was packaged with a Ford-New Holland round baler (model BR 740A, CNH America LLC, Racine, WI).

Structure of Treatments. The treatment structure of each hay harvest was similar; generally, bales were packaged in factorial arrangements of bale diameter (1.5, 1.2, or 0.9 m) and various concentrations of moisture. For the LM, IM, and HM harvests, prestorage bale moistures for interactive treatment combinations ranged from 9.3 to 17.3%, 16.8 to 24.2%, and 26.7 to 46.6%, respectively. All bales were tied with 2 revolutions of net wrap and placed on wooden pallets located outdoors on a dense grass sod.

Temperature Measurements. Each bale was fitted with a thermocouple positioned near its geometric center, and bales were evaluated for maximum internal bale temperature (MAX) during storage. Heating degree days $>30^{\circ}\text{C}$ (HDD) also were computed as the summations of the daily increment by which the internal bale temperature was greater than 30°C . Daily temperature measurements were terminated when bales no longer exhibited any evidence of spontaneous heating; these data for HDD and MAX have been summarized and thoroughly discussed in the companion report (Coblentz and Hoffman, 2009).

Bale Sampling Procedures

Each bale was sampled 3 times, once before storage and twice at the completion of the storage period. Immediately after baling, 0.46-m deep core samples (0.025-m diameter) were taken from the center portion of each bale using a Uni-Forage Sampler (Star Quality Samplers, Edmonton, Alberta, Canada). Samples taken from bales after storage were handled similarly; however, samples were taken from 2 parts of the bale (0.15-m-deep surface layer and the bale core), and then processed and analyzed independently. All samples were dried to constant weight under forced air (50°C), ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) equipped with a 1- or 2-mm screen, and then retained for laboratory evaluation of NDF, ADF, hemicellulose, cellulose, lignin, NDFD, and ruminal in situ disappearance kinetics of NDF.

Laboratory Analyses

Portions of each sample ground through the 1-mm screen were analyzed for NDF, ADF, hemicellulose, cellulose, and lignin, as well as NDFD. Analysis of NDF and other fiber components were conducted sequentially using batch procedures outlined by Ankom

Technology Corp. (Fairport, NY) for an Ankom 200 Fiber Analyzer. Neither sodium sulfite nor α -amylase was included in the NDF solution. Procedures and apparatus for determining NDFD consisted of incubating 0.5-g samples in 125-mL Erlenmeyer flasks containing rumen fluid, buffer media, and macro- and micro-mineral solutions (Goering and Van Soest, 1970). Incubation flasks were purged continuously with CO_2 and maintained in a water bath at 39°C . After 48 h, incubations were terminated by digestion in neutral-detergent solution that included both heat-stable α -amylase and sodium sulfite (Goering and Van Soest, 1970; Mertens, 1992). Before ruminal incubation, rumen fluid was harvested from a nonlactating dairy cow fitted with a ruminal cannula and offered a diet containing 69% alfalfa-grass silage and 30% corn silage, with the balance of the diet consisting of vitamin and mineral supplements.

In Situ Incubation Procedures

Eighteen interactive (bale moisture \times bale diameter) treatments from the HM (10 treatments) and IM (8 treatments) harvests were selected for in situ analysis. All poststorage hays consisted of forage from the bale core only, and each hay treatment was composited over the 3 field replications (bales) before conducting kinetic evaluations. The 18 treatments were selected to provide the best possible distribution across the entire heating continuum represented by IM and HM harvests. Composites of prestorage samples generated from the 10 hays selected from the HM harvest and the 8 hays selected from the IM harvest also were evaluated as (no-heating) controls.

Two nonlactating, 937 ± 35.4 -kg, ruminally cannulated Holstein cows were housed in individual 4.3×8.5 -m pens with concrete floors that were bedded with wood shavings and cleaned regularly. Cannulations (protocol #A-1307) and care of the cows (protocol #A-1339) were approved by the Research Animal Resources Center of the University of Wisconsin. A basal diet consisting of alfalfa/quackgrass hay (14.0% CP, 50.8% NDF, and 36.0% ADF), ground corn, and trace-mineralized salt was offered in equal portions at 0900 and 1500 h at a daily cumulative rate of 1.35% of BW. The basal diet contained 90.3% alfalfa/grass hay, 8.9% ground corn, and 0.8% trace-mineralized salt on an as-fed basis. Cows were allowed a 10-d adaptation period, and then hays were evaluated during two 4-d experimental periods. Following period 1, cows were given a 3-d recovery period before initiating period 2.

In situ procedures were consistent with the standardized techniques described by Vanzant et al. (1998), in which 5-g dried hay samples were sealed in 10×20 -cm Dacron bags (50 ± 10 μm pore size; Ankom Technol-

Table 1. Concentrations of fiber components and 48-h neutral detergent fiber digestibility (NDFD) summarized from 96 round bales of alfalfa-orchardgrass hay from 3 harvests made during 2006 and 2007 near Stratford, Wisconsin

| Item | Harvest ¹ | n ² | Bales, n ³ | Prestorage | | Poststorage surface | | Poststorage core | | | |
|------------------|----------------------|----------------|-----------------------|-------------------|-----------------|---------------------|-----------------|-------------------|----------------------|----------------------|-----------------|
| | | | | Mean ⁴ | SE ⁵ | Mean ⁴ | SE ⁵ | Mean ⁴ | Maximum ⁶ | Minimum ⁷ | SE ⁸ |
| NDF, % | HM | 13 | 39 | 47.8 | 0.20 | 57.0 | 0.23 | 56.5 | 58.7 | 53.7 | 0.54 |
| | IM | 12 | 36 | 47.5 | 0.24 | 53.4 | 0.40 | 52.0 | 54.8 | 48.3 | 1.08 |
| | LM | 7 | 21 | 42.4 | 0.39 | 43.9 | 0.39 | 45.1 | 47.9 | 42.1 | 1.09 |
| ADF, % | HM | 13 | 39 | 32.6 | 0.14 | 39.7 | 0.19 | 39.2 | 41.3 | 37.2 | 0.64 |
| | IM | 12 | 36 | 32.0 | 0.23 | 34.6 | 0.28 | 33.0 | 34.8 | 29.6 | 1.00 |
| | LM | 7 | 21 | 28.3 | 0.13 | 28.4 | 0.19 | 27.2 | 27.8 | 26.1 | 0.46 |
| Hemicellulose, % | HM | 13 | 39 | 15.2 | 0.19 | 17.3 | 0.17 | 17.2 | 20.5 | 14.4 | 0.68 |
| | IM | 12 | 36 | 15.5 | 0.18 | 18.7 | 0.31 | 19.0 | 21.7 | 16.3 | 1.03 |
| | LM | 7 | 21 | 14.1 | 0.34 | 15.5 | 0.33 | 17.9 | 20.7 | 16.0 | 1.11 |
| Cellulose, % | HM | 13 | 39 | 26.3 | 0.08 | 29.7 | 0.14 | 28.8 | 30.0 | 27.8 | 0.52 |
| | IM | 12 | 36 | 25.6 | 0.19 | 27.6 | 0.21 | 26.2 | 27.9 | 24.2 | 0.75 |
| | LM | 7 | 21 | 21.9 | 0.16 | 21.6 | 0.19 | 21.4 | 21.7 | 20.9 | 0.49 |
| Lignin, % | HM | 13 | 39 | 5.54 | 0.506 | 8.15 | 0.091 | 8.80 | 9.99 | 7.41 | 0.310 |
| | IM | 12 | 36 | 5.60 | 0.087 | 6.45 | 0.107 | 5.96 | 6.50 | 4.70 | 0.296 |
| | LM | 7 | 21 | 5.42 | 0.151 | 5.10 | 0.111 | 5.41 | 5.95 | 5.00 | 0.140 |
| NDFD, % of NDF | HM | 13 | 39 | 48.7 | 0.41 | 48.1 | 0.41 | 47.7 | 50.9 | 43.1 | 1.79 |
| | IM | 12 | 36 | 48.3 | 0.55 | 45.9 | 0.72 | 48.3 | 50.8 | 46.7 | 1.85 |
| | LM | 7 | 21 | 46.7 | 0.37 | 48.1 | 0.71 | 47.0 | 47.9 | 46.0 | 1.98 |

¹Harvest: HM = high moisture (26.7 to 46.6%); IM = intermediate moisture (16.8 to 24.2%); and LM = low moisture (9.3 to 17.3%).

²Number of interactive treatments during each harvest. Harvest HM contained one baling treatment made at 26.7% moisture at the 0.9-m bale diameter only, whereas LM contained a dry control made at 9.3% moisture and at the 1.2-m bale diameter only. These additional treatments were made only at one diameter because insufficient forage was available to complete the entire factorial arrangement of treatments (bale diameters) at these moisture concentrations. Each interactive treatment represents the mean of 3 bales.

³Total number of bales made per harvest.

⁴Overall mean of all interactive bale moisture \times bale diameter treatments.

⁵Standard error of the overall mean of all interactive bale moisture \times bale diameter treatments.

⁶Maximum value across all interactive treatments.

⁷Minimum value across all interactive treatments.

⁸Standard error of the interactive mean.

ogy, Corp.), and then suspended in the ventral rumen for 3, 6, 9, 12, 24, 36, 48, 72, or 96 h. Before insertion into the rumen, all Dacron bags were placed in 35- \times 50-cm mesh bags and incubated in tepid water (39°C) for 20 min. After incubation, samples were rinsed immediately in a top-loading washing machine (Coblentz et al., 1997b; Vanzant et al., 1998). For all selected hays, additional bags were preincubated and rinsed without ruminal incubation, creating a 0-h incubation time. After machine rinsing, residues were dried at 50°C, equilibrated with the atmosphere in the laboratory (Vanzant et al., 1996), and then weighed to determine residual DM. To determine NDF disappearance kinetics, 0.5-g subsamples of the air-equilibrated residues from each Dacron bag were digested in NDF solution using the Ankom procedures described previously; no heat-stable α -amylase or sodium sulfite were included in the neutral-detergent solution. Using these procedures, the entire study comprised 800 Dacron bags. Allocation of bags and time periods was identical across all animals and experimental periods. Therefore, for each of the 4 animal \times period combinations, there were 200 Dacron bags in total (20 hays \times 10 time periods); of these, 180 bags were incubated within the ventral rumen and

20 represented the 0-h bags that were presoaked and rinsed without ruminal incubation.

The percentage of NDF remaining at each incubation time was fitted to the nonlinear regression model of Mertens and Loften (1980) using PROC NLIN (SAS Institute, 1990), which partitions NDF into 3 fractions based on relative susceptibility to ruminal disappearance. Fractions A, B, and C are defined as the portions of NDF disappearing at a rate too fast to measure, disappearing at a measurable rate, or unavailable in the rumen, respectively. Fractions B and C, as well as lag time and K_d were estimated directly from the regression model. Fraction A was calculated as 100% - (B + C), and effective ruminal disappearance of NDF was calculated as $A + B \times [K_d / (K_d + K_p)]$ (Ørskov and McDonald, 1979), where K_p = passage rate (0.06/h; Hoffman et al., 1993). An independent ruminal NDF decay curve was fitted for each combination of animal, period, and forage, resulting in a total of 80 curves for the entire project.

Statistics

Regressions of NDF, ADF, Hemicellulose, Cellulose, Lignin, and NDFD on HDD and MAX.

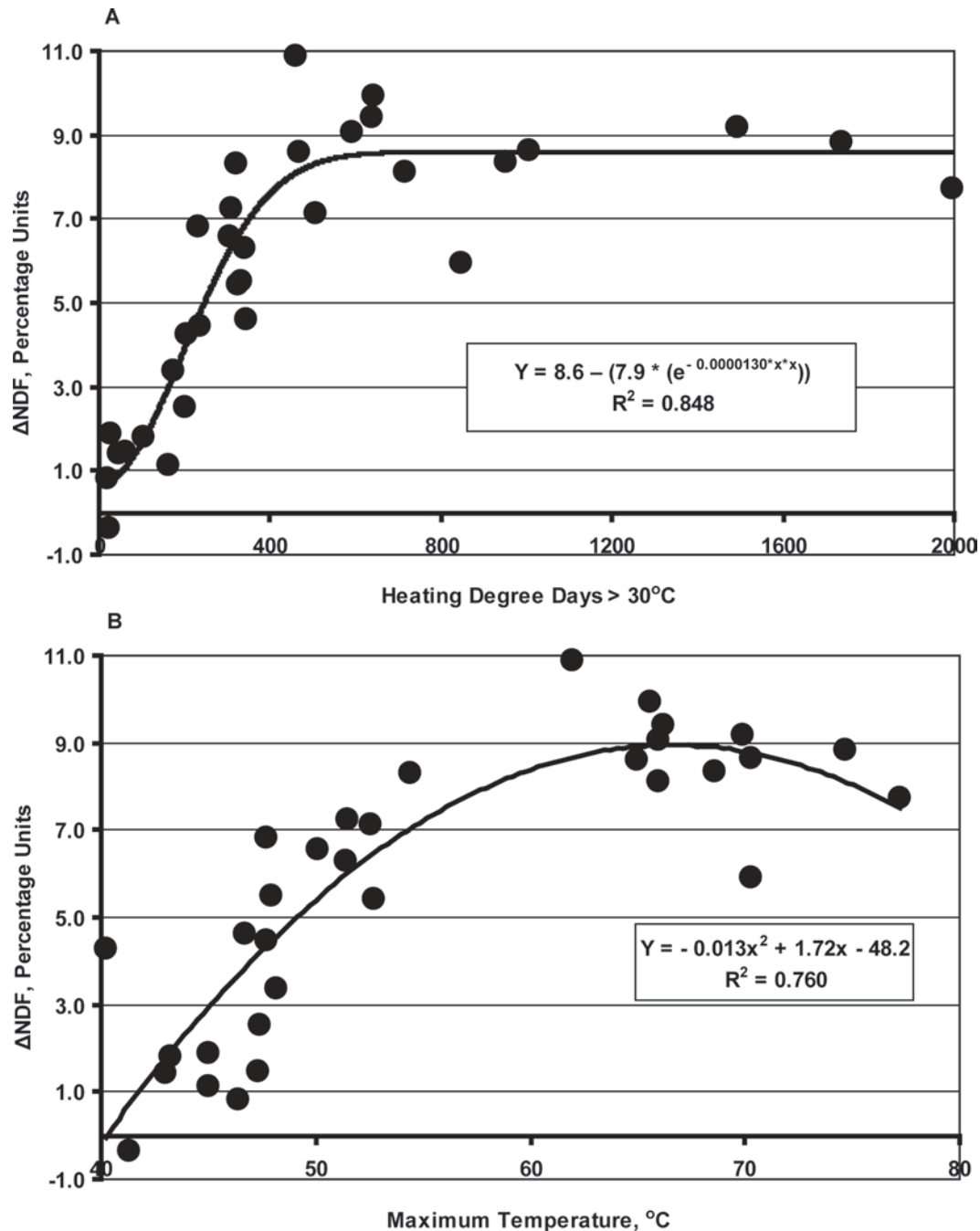


Figure 1. Changes in concentrations of NDF (poststorage – prestorage; ΔNDF) as affected by heating degree days >30°C (A) and maximum internal bale temperature (B). The mean prestorage concentration of NDF (weighted based on the number of treatments from the high-, intermediate-, and low-moisture harvests) was 46.5%, which corresponds generally to $\Delta\text{NDF} = 0$ on the y-axis.

Although data for each individual harvest were analyzed independently (data not shown), we concluded that the randomized fixed effects (bale moisture and diameter) evaluated throughout these hay harvests affected fiber composition and fiber digestibility primarily through their close relationship with heating characteristics (Coblentz and Hoffman, 2009). Originally, it was our intention to analyze and report on each harvest as an

independent randomized fixed-effect trial, and then combine the data from the 3 harvests by regressing response variables on indices of spontaneous heating (HDD and MAX). However, these statistical approaches were redundant, largely because varying bale moisture and diameter within each individual harvest created a range of conditions that were more or less favorable for spontaneous heating and the subsequent retention

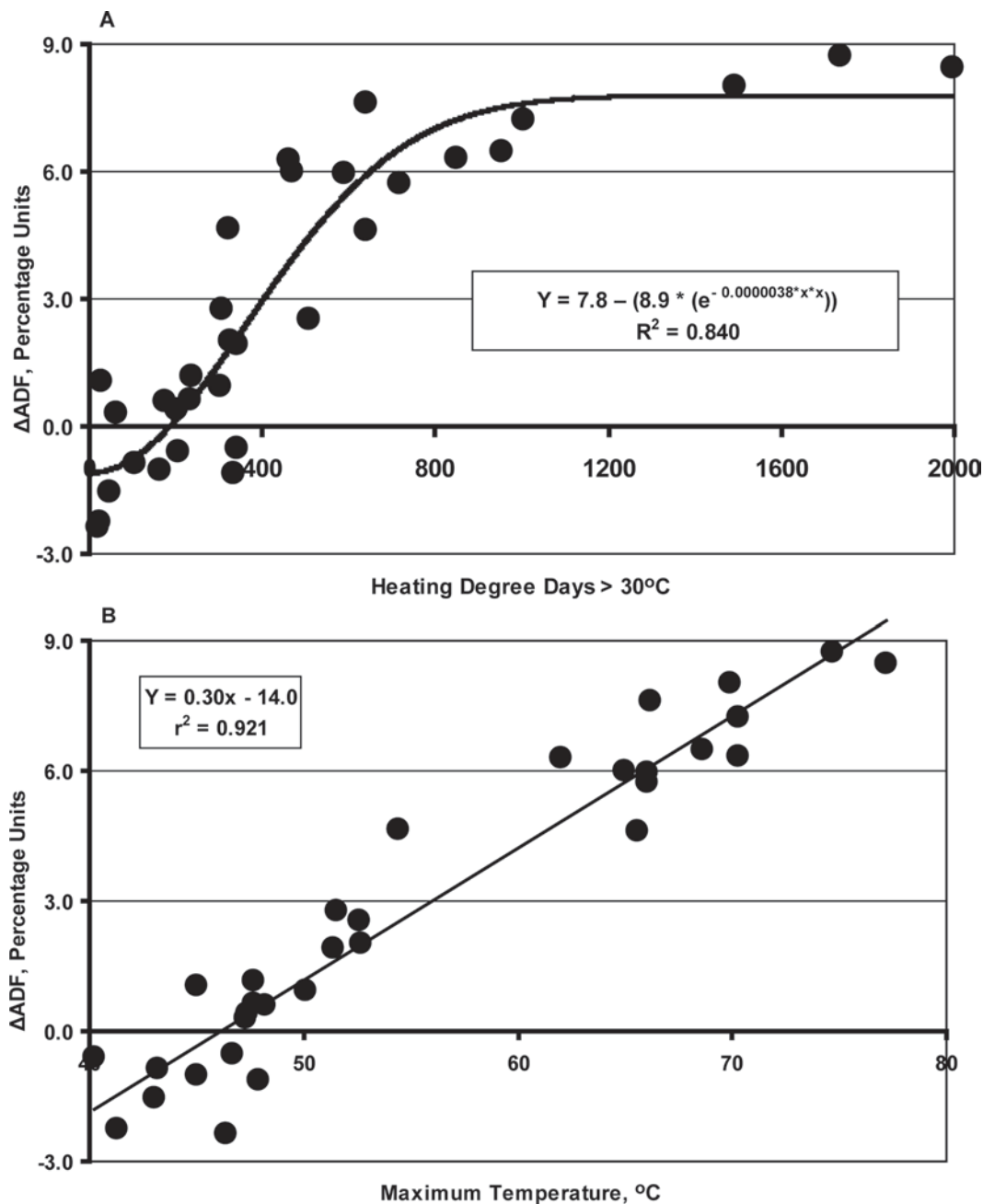


Figure 2. Changes in concentrations of ADF (poststorage – prestorage; ΔADF) as affected by heating degree days >30°C (A) and maximum internal bale temperature (B). The mean prestorage concentration of ADF (weighted based on the number of treatments from the high-, intermediate-, and low-moisture harvests) was 31.4%, which corresponds generally to $\Delta\text{ADF} = 0$ on the y-axis.

of heat within each bale. Therefore, to simplify the presentation of results, the ANOVA for each individual harvest have been omitted, and all data are pooled and analyzed collectively by direct regression on measures of spontaneous heating (HDD or MAX).

Before conducting regression analyses, poststorage concentrations of NDF, ADF, hemicellulose, cellulose, lignin, and NDFD from the bale core were normalized as a simple mathematical difference resulting from storage

(poststorage – prestorage; ΔNDF , ΔADF , ΔHEMI , ΔCELL , ΔLIG , and ΔNDFD , respectively), where positive and negative values indicate increased or decreased concentrations, respectively. Normalization of data was required to account for relatively minor differences in prestorage concentrations of fiber components (Table 1) across the 3 harvests. These baseline-adjusted response variables were then regressed against HDD and MAX using nonlinear regression models (PROC

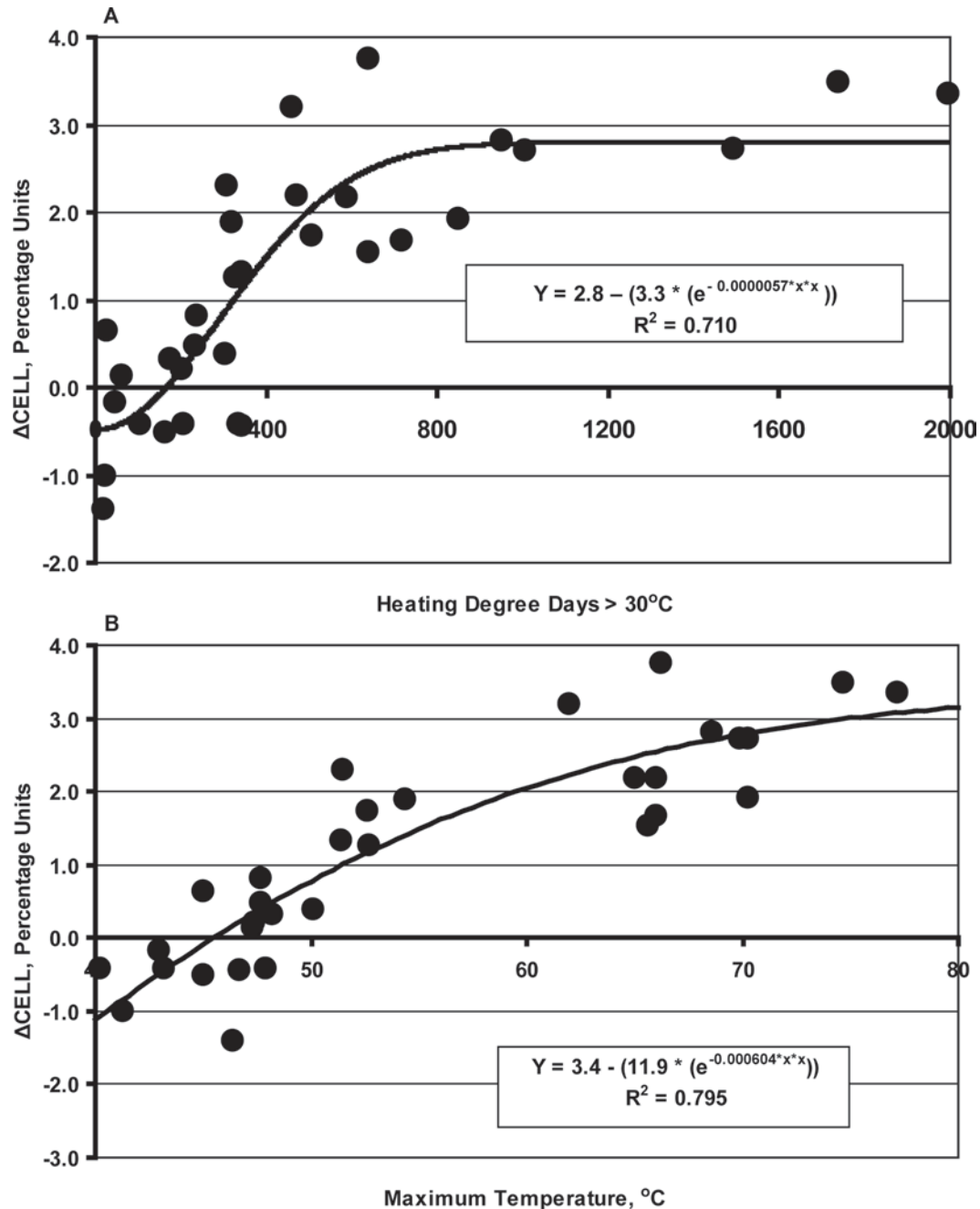


Figure 3. Changes in concentrations of cellulose (poststorage – prestorage; ΔCELL) as affected by heating degree days >30°C (A) and maximum internal bale temperature (B). The mean prestorage concentration of cellulose (weighted based on the number of treatments from the high-, intermediate-, and low-moisture harvests) was 25.1%, which corresponds generally to ΔCELL = 0 on the y-axis.

NLIN; SAS Institute, 1990) of the general form $Y = b \times (e^{-kx}) - a$, if ΔNDF, ΔADF, ΔCELL, ΔLIG, or ΔNDFD became negative with spontaneous heating, or $Y = a - (b \times e^{-kx})$, if they became positive. For these model assessments, k was the rate constant, x was the independent variable (HDD or MAX), and a and b were parameters determined directly by the regression model. For these nonlinear regression models, the inde-

pendent variables (HDD or MAX) also were squared in an attempt to improve fit. Because of the distribution of the MAX measurement, nonlinear regression models generally were more difficult to apply when MAX was the independent variable. For hay experiments, MAX = 0°C is nonsensical, and the overall range for MAX in the present studies was confined from 40.2 to 77.2°C, with no data distributed between MAX = 0

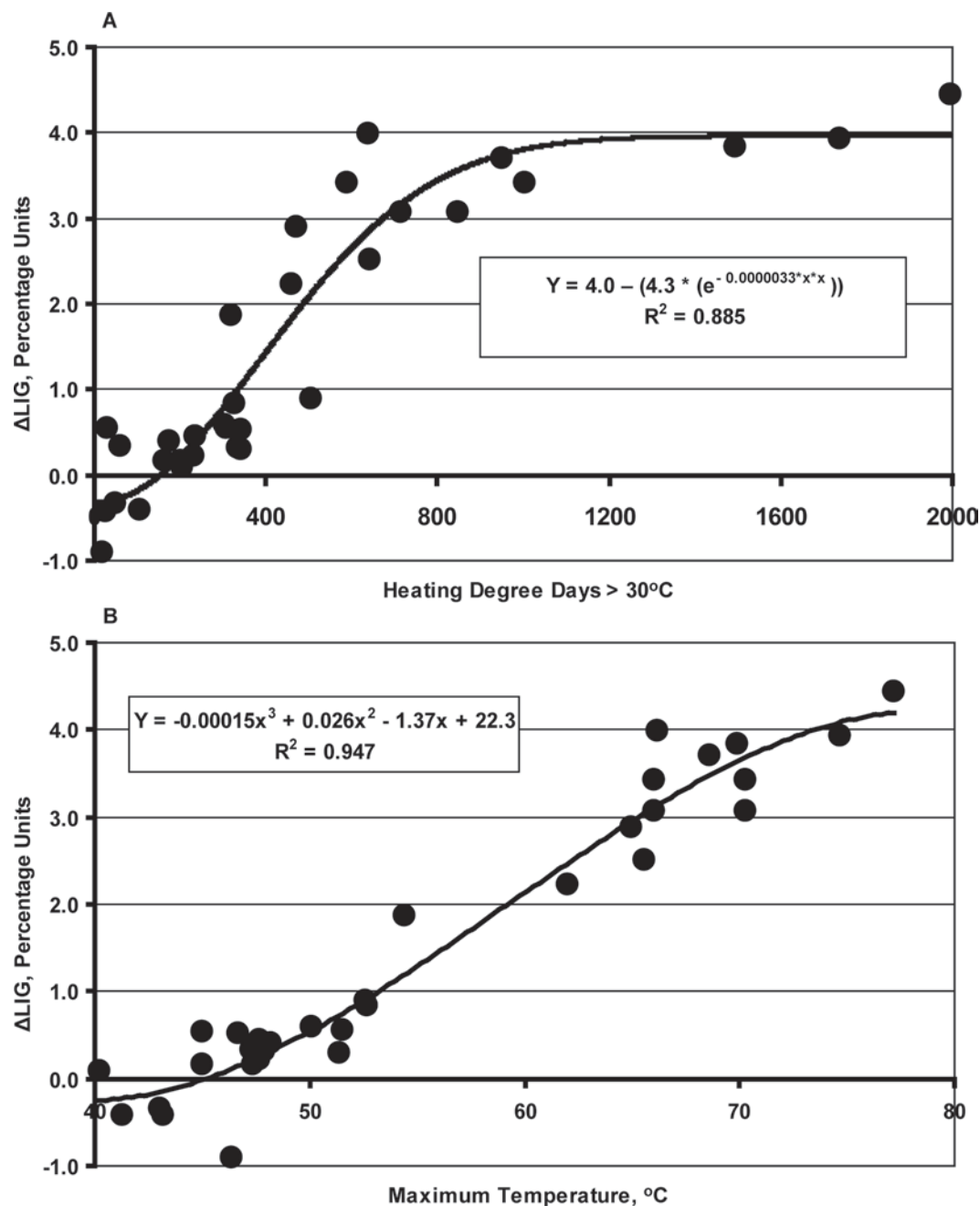


Figure 4. Changes in concentrations of ADL (poststorage – prestorage; ΔLIG) as affected by heating degree days >30°C (A) and maximum internal bale temperature (B). The mean prestorage concentration of ADL (weighted based on the number of treatments from the high-, intermediate-, and low-moisture harvests) was 5.54%, which corresponds generally to ΔLIG = 0 on the y-axis.

and 40.2°C. Four other polynomial regression models assessing linear, quadratic, cubic, and quartic responses to HDD or MAX also were evaluated by PROC REG (SAS Institute, 1990).

The selection of the most appropriate model for kinetic characteristics of ruminal NDF disappearance was assessed similarly. Fractions A, B, and C, as well as lag time, K_d , and effective degradability of NDF were

subjected to ANOVA procedures, thereby generating a mean response for each kinetic parameter over the 4 combinations of animal and period. As described previously for fiber components, kinetic characteristics were then normalized across hay harvests by expressing each kinetic parameter as a mathematical (baseline-adjusted) difference resulting from storage (poststorage – prestorage; ΔA, ΔB, ΔC, ΔLAG, ΔK_d, and

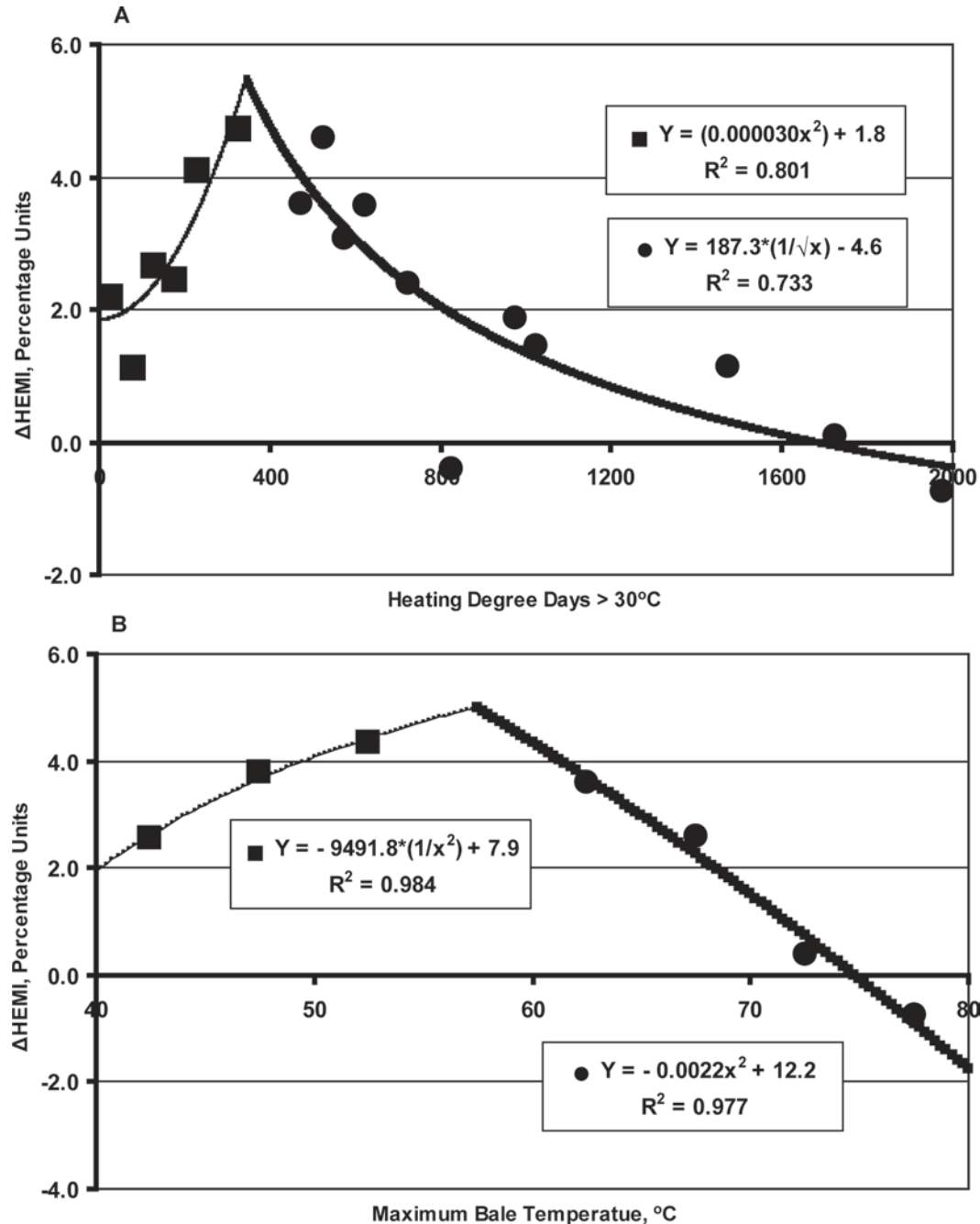


Figure 5. Changes in concentrations of hemicellulose (poststorage – prestorage; ΔHEMI) as affected by heating degree days (HDD) > 30°C (A) and maximum internal bale temperature (MAX; B). The mean prestorage concentration of hemicellulose (weighted based on the number of treatments from the high-, intermediate-, and low-moisture harvests) was 15.1%, which corresponds generally to $\Delta\text{HEMI} = 0$ on the y-axis. To reduce variability, data were grouped in increments of 50 HDD and 5°C (MAX) before conducting regression analysis. Independent regressions were conducted for the ascending (■, thin line) and descending (●, thick line) portions of each response; in panel A, the intersection of the 2 regression lines occurred at 347 HDD, whereas for MAX (panel B) it occurred at 57.5°C.

ΔDEG , respectively). Generally, selection of the most appropriate model was based on the coefficient of determination (r^2 or R^2). Polynomial regression models were not selected if the coefficient or slope for the highest ordered term did not differ from zero, or if the model included an illogical inflection in the regression

curve that could not be explained biologically. It should be noted clearly that these regression techniques are meant only to describe changes within each kinetic parameter as a function of HDD and MAX; it should not be inferred that these procedures are sufficiently rigorous to extrapolate beyond the context of this data

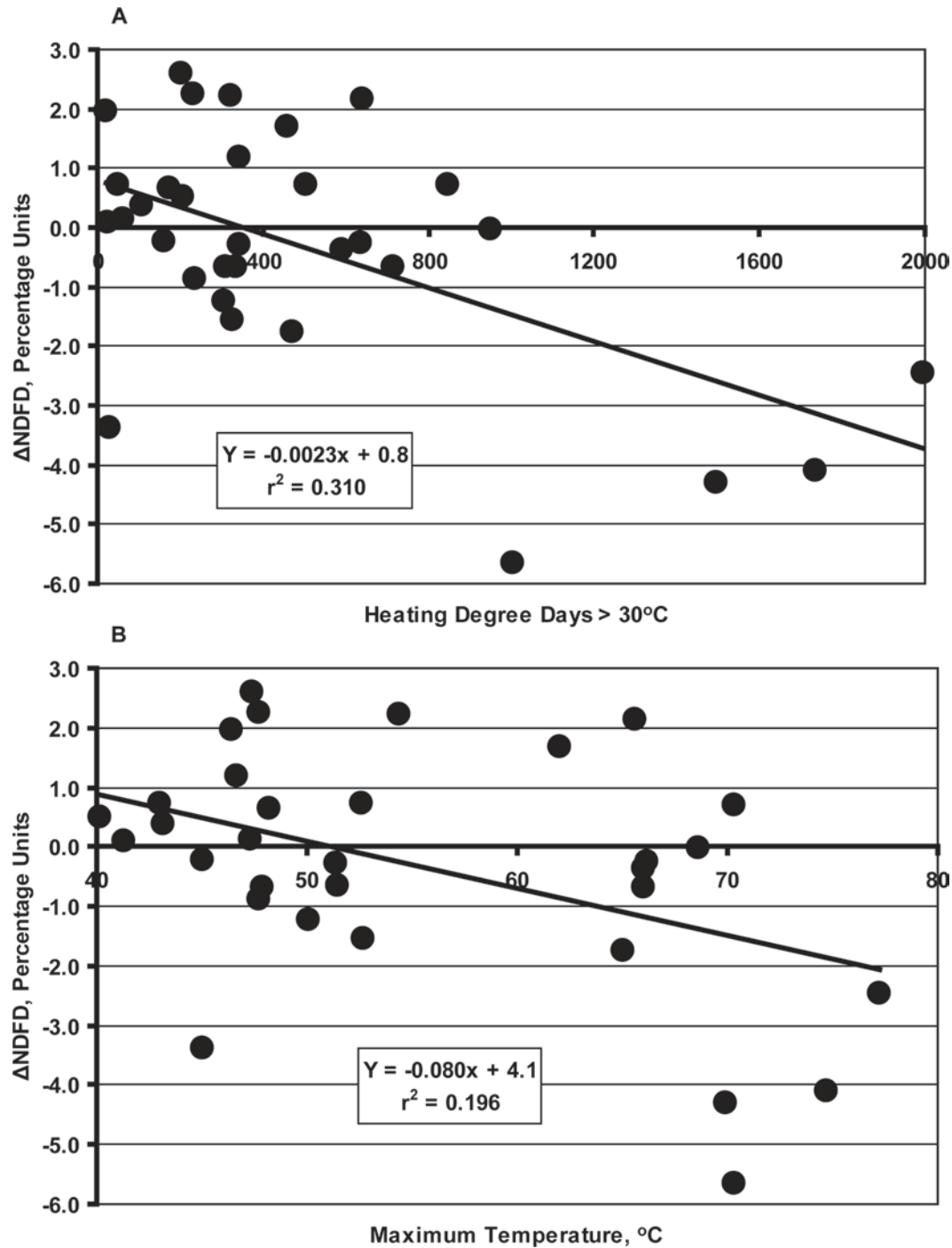


Figure 6. Changes in concentrations of 48-h NDF digestibility (poststorage – prestorage; Δ NDFD) as affected by heating degree days >30°C (A) and maximum internal bale temperature (B). The mean prestorage concentration of NDFD (weighted based on the number of treatments from the high-, intermediate-, and low-moisture harvests) was 48.1% of NDF, which corresponds generally to Δ NDFD = 0 on the y-axis.

set, or to serve as a direct basis for estimating values for unknown forages.

Generally, these regression procedures were suitable for explaining responses to spontaneous heating for all response variables except Δ HEMI. Initially, plots of

Δ HEMI on HDD or MAX were highly scattered, but revealed an ascending pattern at relatively low HDD or MAX and a descending relationship at more extreme spontaneous heating. To best explain these responses, data were grouped over increments of 50 HDD and 5°C

Table 2. In situ disappearance kinetics of NDF for 20 baling treatments selected from the high- and intermediate-moisture harvests¹

| Hay number | Bale diameter, m | Initial bale moisture, % | HDD >30°C | Maximum temperature, °C | Fraction, % of NDF | | | Lag time, h | K _d , /h | Effective degradability, ² % of NDF |
|-----------------------|-----------------------------------|--------------------------|-----------|-------------------------|--------------------|------|------|-------------|---------------------|--|
| | | | | | A | B | C | | | |
| High moisture | | | | | | | | | | |
| 1 | Prestorage composite ³ | 0 | — | — | 14.0 | 48.8 | 37.2 | 2.17 | 0.104 | 44.8 |
| 2 | 0.9 | 26.7 | 321 | 54.4 | 18.6 | 49.3 | 32.1 | 1.98 | 0.082 | 46.8 |
| 3 | 0.9 | 38.7 | 470 | 65.0 | 19.9 | 45.6 | 34.5 | 2.72 | 0.076 | 45.1 |
| 4 | 0.9 | 41.9 | 590 | 66.0 | 22.5 | 48.3 | 29.2 | 2.85 | 0.062 | 46.9 |
| 5 | 1.2 | 30.9 | 641 | 65.6 | 21.0 | 47.7 | 31.3 | 3.29 | 0.077 | 47.6 |
| 6 | 1.5 | 32.1 | 716 | 66.0 | 20.9 | 46.3 | 32.8 | 2.16 | 0.058 | 43.2 |
| 7 | 1.2 | 39.4 | 952 | 68.6 | 20.0 | 47.3 | 32.7 | 2.07 | 0.066 | 44.7 |
| 8 | 1.2 | 43.5 | 1,005 | 70.3 | 20.9 | 45.3 | 33.8 | 3.17 | 0.072 | 45.5 |
| 9 | 1.5 | 38.7 | 1,494 | 69.9 | 20.6 | 47.1 | 32.3 | 1.93 | 0.064 | 44.9 |
| 10 | 1.5 | 40.1 | 1,737 | 74.7 | 21.7 | 47.2 | 31.0 | 2.02 | 0.064 | 46.1 |
| 11 | 1.5 | 46.6 | 1,997 | 77.2 | 23.6 | 43.8 | 32.5 | 2.88 | 0.063 | 45.9 |
| SEM | | | | | 0.77 | 0.80 | 0.46 | 0.440 | 0.0059 | 0.73 |
| Intermediate moisture | | | | | | | | | | |
| 12 | Prestorage composite ⁴ | 0 | — | — | 16.0 | 46.1 | 37.9 | 0.87 | 0.094 | 43.8 |
| 13 | 0.9 | 17.1 | 29 | 45.0 | 15.9 | 45.2 | 39.0 | 1.55 | 0.100 | 43.9 |
| 14 | 1.2 | 17.5 | 62 | 47.3 | 14.8 | 49.0 | 36.1 | 1.56 | 0.090 | 43.6 |
| 15 | 1.2 | 18.9 | 175 | 48.2 | 14.5 | 47.7 | 37.8 | 2.02 | 0.094 | 43.3 |
| 16 | 1.5 | 16.8 | 203 | 47.4 | 14.4 | 49.7 | 35.9 | 1.52 | 0.085 | 43.4 |
| 17 | 1.5 | 22.0 | 304 | 50.1 | 14.2 | 50.0 | 35.7 | 1.71 | 0.084 | 42.9 |
| 18 | 1.5 | 20.3 | 308 | 51.5 | 15.8 | 48.9 | 35.3 | 2.45 | 0.083 | 44.1 |
| 19 | 1.5 | 24.2 | 326 | 52.7 | 15.6 | 47.0 | 37.4 | 1.39 | 0.079 | 42.1 |
| 20 | 1.2 | 22.8 | 506 | 52.6 | 16.9 | 48.9 | 34.2 | 0.85 | 0.065 | 42.3 |
| SEM | | | | | 1.03 | 1.15 | 0.39 | 0.386 | 0.0087 | 1.03 |

¹HDD >30°C = heating degree days accumulated during bale storage; fraction A = fraction of total NDF pool disappearing at a rate too rapid to measure; fraction B = fraction of total NDF pool disappearing at a measurable rate; fraction C = fraction of total NDF pool unavailable in the rumen; and K_d = fractional rate constant.

²Calculated as $A + B \times [(K_d + K_p)/K_d]$, where K_p was the ruminal passage rate, which was arbitrarily set at 0.06/h (Hoffman et al., 1993).

³Composite equally weighted with sample obtained immediately after baling from hays 2 through 11.

⁴Composite equally weighted with sample obtained immediately after baling from hays 13 through 20.

for MAX to reduce scatter. After grouping, independent regressions were conducted for the ascending and descending portions of each response for Δ HEMI over HDD or MAX. In addition, a wider range of regression models was considered to obtain the best fit; these included dropping the linear term from quadratic regression models, and using alternate data transformation terms, such as $1/\sqrt{\text{HDD}}$ or $1/\text{MAX}^2$. Throughout all regression analyses, significance was declared at $P \leq 0.05$, unless otherwise indicated.

RESULTS AND DISCUSSION

Regressions of Changes in Concentrations of Fiber Components and NDFD on HDD and MAX

NDF, ADF, Cellulose, and Lignin. For regressions of Δ NDF (Figure 1A), Δ ADF (Figure 2A), Δ CELL (Figure 3A), and Δ LIG (Figure 4A) on HDD, a nonlinear model in which the independent variable (HDD) was squared was most effective in relating changes in fiber components to HDD. In each case, concentrations of these fiber components increased rapidly with HDD, but then became asymptotic between approximately

600 and 1,200 HDD. Coefficients of determination (R^2) ranged from 0.885 for Δ LIG to 0.710 for Δ CELL, but indicated a relatively close relationship in each case.

Regressions of Δ NDF (Figure 1B), Δ ADF (Figure 2B), and Δ LIG (Figure 4B) on MAX were best explained with quadratic, linear, and cubic models, respectively. Relatively high coefficients of determination ($R^2 = 0.760$ to 0.947) were exhibited in each case, indicating that MAX was an excellent predictor of changes in concentrations of these fiber components. In contrast, the regression of Δ CELL on MAX (Figure 3B) was best fitted to the nonlinear model with the independent variable squared; however, the coefficient of determination also was relatively high ($R^2 = 0.795$). Within these relationships, Δ ADF, Δ CELL, and Δ LIG all became increasingly positive over the entire range of MAX observed throughout our harvests. In contrast, a negative inflection in the quadratic regression curve for Δ NDF suggests that concentrations of NDF may decline slightly at relatively high MAX (>66°C). Declining concentrations of NDF suggest that portions of the NDF matrix, such as hemicellulose, may become reactive and lose their normal analytical properties (Van Soest, 1982).

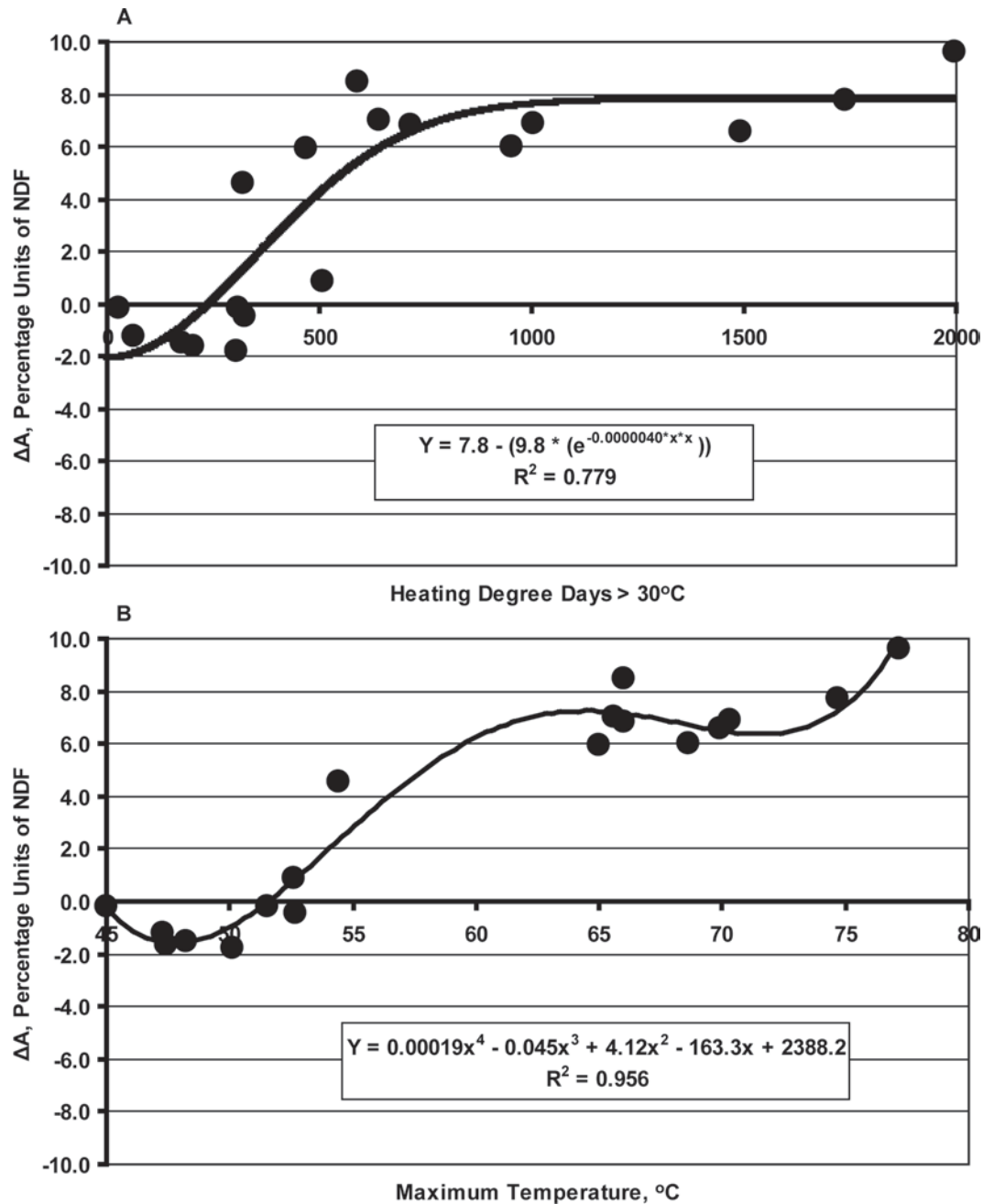


Figure 7. Changes in percentages of NDF disappearing from Dacron bags at a rate too rapid to measure (poststorage – prestorage; ΔA) as affected by heating degree days >30°C (A) and maximum internal bale temperature (B). The mean prestorage concentration of fraction A (weighted based on the number of treatments from the high- and intermediate-moisture harvests) was 14.9% of NDF, which corresponds generally to $\Delta A = 0$ on the y-axis.

Previously, concentrations of NDF, ADF, and lignin have been related linearly to HDD, MAX, and average internal bale temperature for bermudagrass hays (Coblentz et al., 2000), and similar linear relationships have been established for NDF and ADF regressed on HDD for alfalfa hays (Coblentz et al., 1996). As observed in the present studies, these regressions were

characterized by high r^2 statistics, which ranged up to 0.952 for a linear relationship between NDF and HDD within heated bermudagrass hays (Coblentz et al., 2000). Within the present study, the curvilinear nature for many of our regression relationships is most likely a function of greater spontaneous heating incurred in large-round bales. Accumulations of HDD for interactive

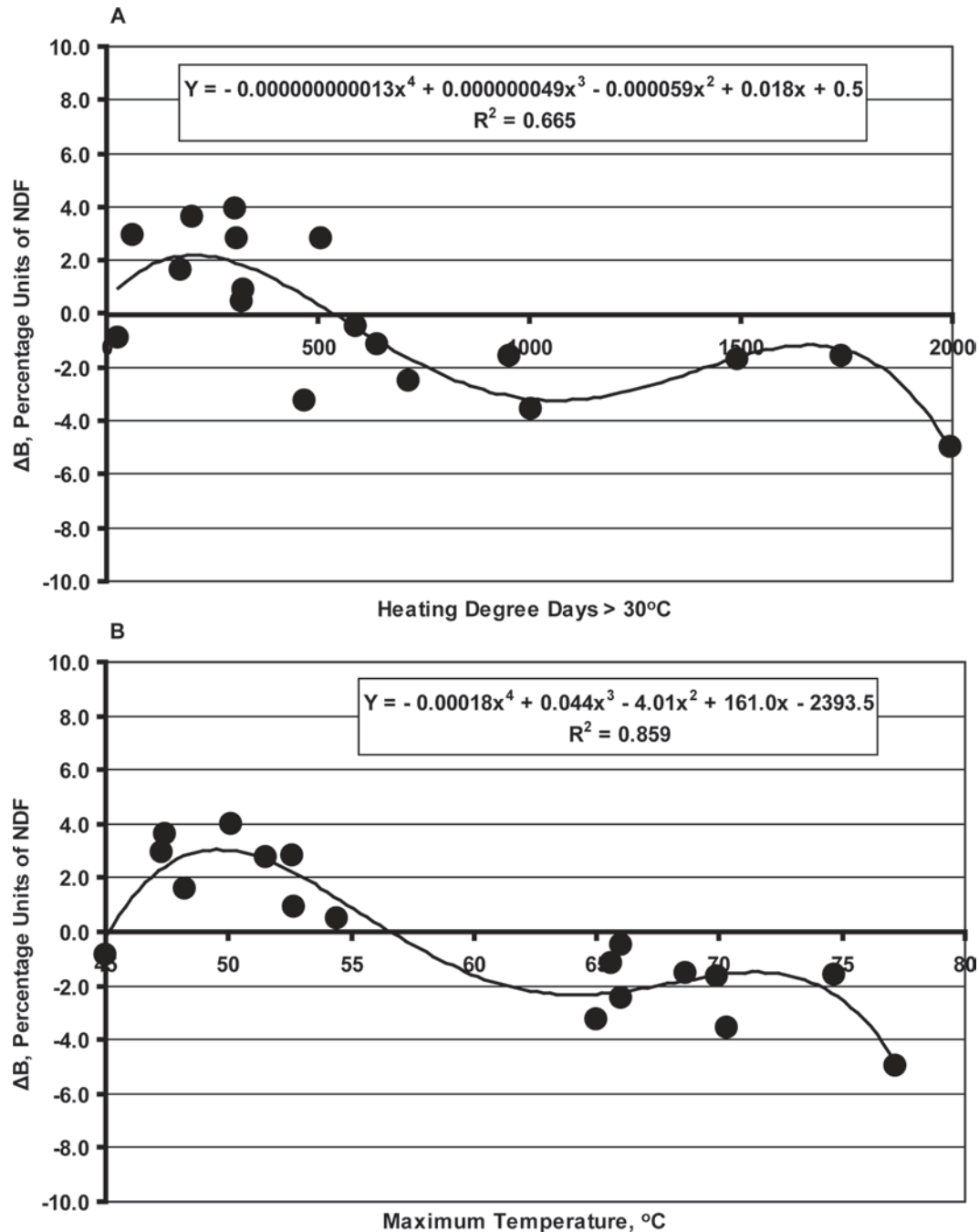


Figure 8. Changes in percentages of NDF disappearing from Dacron bags at a measurable rate (poststorage – prestorage; ΔB) as affected by heating degree days >30°C (A) and maximum internal bale temperature (B). The mean prestorage concentration of fraction B (weighted based on the number of treatments from the high- and intermediate-moisture harvests) was 47.6% of NDF, which corresponds generally to $\Delta B = 0$ on the y-axis.

treatments in the present study ranged as high as 1,997 HDD (Coblentz and Hoffman, 2009), which was approximately 5 and 6 times the maximum accumulations reported for small rectangular bales of bermudagrass packaged at 32.5% moisture (Coblentz et al., 2000) and alfalfa packaged at 29.7% moisture (Coblentz et

al., 1996), respectively. Because concentrations of fiber components increase primarily by indirect mechanisms during bale storage, the asymptotic nature of ΔNDF , ΔADF , $\Delta CELL$, and ΔLIG within regressions on HDD may also indicate near exhaustion of substrate pools for microbial respiration, thereby limiting the indirect

concentrating effect on fiber components. It also should be noted that under conditions favoring more extreme spontaneous heating, such as those observed for the HM harvest (Coblentz and Hoffman, 2009), accumulation of lignin could occur directly via formation of Maillard products (Van Soest, 1982).

Hemicellulose. Regressions of ΔHEMI on HDD or MAX were unique relative to all other response variables (Figures 5A and 5B). In both cases, data were scattered, but indicated a distinctly ascending pattern at low-to-modest spontaneous heating, followed by a descending pattern with more extreme heating. After data were grouped in 50 HDD increments to reduce scatter, the ascending portion of the regression on HDD was fit with a quadratic model without the linear term ($R^2 = 0.801$), and the descending portion of the response was best fit with an alternative model [$Y = 187.3(1/x) - 4.6$; $R^2 = 0.733$]. Intersection of these 2 regression responses occurred at approximately 347 HDD. Although the specific regression models varied for relationships with MAX, the diverse ascending and descending aspects of the overall response were similar, yielding an intersection of regression lines at 57.5°C. Generally, these diverse responses over a wide range of spontaneous heating are consistent with other work. Coblentz et al. (2000) reported a positive linear relationship between concentrations of hemicellulose and various measures of spontaneous heating for small rectangular bales of heated bermudagrass hays that incurred a maximum of 327 HDD, based on a 35°C threshold. Conversely, Goering et al. (1973) reported that concentrations of hemicellulose declined rapidly with temperature when orchardgrass forages were hydrated to approximately 53% moisture and then heated artificially for 24 h at 40, 60, 80, or 100°C. Throughout our 3 hay harvests, concentrations of hemicellulose likely increased indirectly via respiration of nonfiber compounds until the accumulation of HDD or MAX approached 347 HDD or 57.5°C, respectively. With more extreme heating, hemicellulose likely became an active participant in Maillard or other reactions. Because damaged carbohydrates cease to retain their undamaged analytical properties and are often recovered as lignin (Van Soest, 1982), concentrations of hemicellulose then declined at high HDD or MAX.

NDFD. Unlike all fiber components, regressions of ΔNDFD on HDD (Figure 6A) or MAX (Figure 6B) were best fit by simple linear models with relatively low coefficients of determination ($r^2 = 0.310$ and 0.196, respectively). Although the negative slopes associated with these models differed from zero ($P \leq 0.011$), they were heavily influenced by 4 negative values of ΔNDFD that were specifically confined to the most extreme accumulations of HDD or greatest MAX. Without these

responses at the most extreme levels of spontaneous heating, there would have been no statistical relationship between ΔNDFD and either HDD ($P = 0.950$) or MAX ($P = 0.920$). These regressions suggest that digestibility of fiber is largely unaffected by spontaneous heating, except in cases where baling moisture and other storage factors are grossly mismanaged, resulting in excessive spontaneous heating.

Previous studies have reported conflicting relationships between spontaneous heating and in vivo digestibility of NDF. For bermudagrass hays accumulating 5 to 401 HDD during storage based on a 35°C threshold, NDF digestibility declined linearly when hays were offered to growing wethers (McBeth et al., 2001); however, this relationship was characterized by a relatively limited range (60.4 to 66.5%) and a relatively poor coefficient of determination ($r^2 = 0.30$). In contrast, Turner et al. (2004) reported a tendency for increased in vivo digestibilities of NDF in growing steers offered tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] hays that heated modestly during storage. Similarly, Broderick et al. (1993) reported increased in vivo digestibility of NDF for steam-treated (100 to 110°C for 0.75 h) alfalfa-hay-based diets offered to lactating dairy cows compared with diets containing unheated hays. In each of these studies, interpretation is complicated somewhat by the conditions of ad-libitum voluntary intake that were established to meet experimental objectives. In reality, this is a procedural compromise that allows in vivo assessment with procedures that resemble most closely production situations, but actually confound estimates of digestibility because of the positive relationship between voluntary intake and rate of passage (Cochran and Galyean, 1994).

Generally, our results suggest that NDFD was affected only marginally by the wide range of baling treatments and heating characteristics generated throughout the LM, IM, and HM harvests. Potentially, this has implications with respect to the prediction of truly digestible fiber, and subsequently, energy density (total digestible nutrients) within forages by the summative approach (Weiss et al., 1992; NRC, 2001). Specifically, estimation of truly digestible fiber for heated forages would be sensitive primarily to changes in NDF concentrations when estimated by NDFD, but the alternate lignin-based calculation would be sensitive to changes in concentrations of both NDF and lignin that occur because of spontaneous heating.

In Situ Disappearance Kinetics of NDF

Fractions A, B, and C, as well as discrete lag time, K_d , and effective ruminal degradability are compiled in Table 2 for the 18 interactive combinations of bale

moisture and density sampled from the bale core, plus prestorage controls from both the IM and HM harvests. Selections were weighted more heavily for HDD accumulations ranging between 29 and 641 d (12 hays), which is probably the most relevant part of the overall range with respect to commercial hay production.

Fractions A, B, and C. Fraction A is defined as that portion of the total NDF pool disappearing at a rate too fast to measure. For other forage components, such as DM or CP, fraction A is often associated closely with solubility in water, and may even be defined as the soluble or immediately soluble fraction (Hoffman et al., 1993; Vanzant et al., 1996; Broderick and Cochran, 2000). For NDF disappearance kinetics, interpretation of fraction A is complicated by the theoretical insolubility of NDF in water (Van Soest, 1982), which implies that fraction A should comprise only a negligible percentage of the total NDF pool. In practice, this concept has been observed inconsistently. Numerous studies have reported measurable percentages of the total NDF pool within fraction A, which implies incomplete recovery of NDF following machine-washing of 0-h bags. Utilizing rinsing procedures similar to those in the present study, fraction A has often comprised only a small percentage of NDF, ranging from 5.2 to 7.5% for common crabgrass [*Digitaria ciliaris* (Retz.) Koel.] (Ogden et al., 2005), 0.9 to 5.9% for headed tall fescue hays (Turner et al., 2004), and 3.1 to 4.0% for heated bermudagrass hays (McBeth et al., 2003). Often, it has been suggested that these small percentages of the total NDF pool are associated with direct losses of small particles from the Dacron bags during rinsing, which is a criticism of the in situ procedure generally (Broderick, 1994; Broderick and Cochran, 2000). Other studies, particularly those evaluating cool-season grasses, have reported considerably greater percentages of NDF within fraction A. Hoffman et al. (1993) reported concentrations of fraction A for timothy (*Phleum pratense* L.) harvested at the second-node, boot, and full-inflorescence stages of growth to be 29.4, 11.8, and 13.4% of NDF, respectively. Similarly, Flores et al. (2007) reported a mean fraction A of 14.6% of NDF from pregrazed and postgrazed autumn-stockpiled tall fescue forages sampled from December through February in Arkansas. In the present study, fraction A for hays sampled immediately after baling from the IM and HM harvests comprised 16.0 and 14.0% of NDF, respectively (Table 2). Although questions remain about the proper interpretation of this fraction, the IM and HM harvests included 22 and 31% vegetative orchardgrass; therefore, relatively high concentrations of fraction A are not necessarily inconsistent with past work.

Regressions of ΔA on measures of spontaneous heating were best fit by a nonlinear model in which HDD

were squared (Figure 7A), and by a quartic model for MAX (Figure 7B). In both cases, ΔA declined minimally at relatively low increments of heating, but then increased to nearly 10 percentage units of NDF in the most severely heated bales. Although the overall fit for the quartic regression model for MAX ($R^2 = 0.956$) was substantially better than the nonlinear model for HDD ($R^2 = 0.779$), both regressions indicate that fraction A increased with spontaneous heating, especially when HDD or MAX exceed thresholds typically attained within small rectangular bales. Previously, McBeth et al. (2003) reported no change in fraction A within heated bermudagrass hays packaged in small rectangular bales that incurred from 5 to 401 HDD during storage. Our results over this range of heating are largely comparable, with ΔA ranging from -0.1 to -1.8 percentage units when heating was limited to 308 HDD or less (Figure 7A). It remains unclear why ΔA became increasingly positive as spontaneous heating became more extreme. Possibly, this could be the result of physical changes to hays, such as brittleness, that may affect particle-size distribution following grinding, and the subsequent migration of minute forage particles from Dacron bags during rinsing.

For ΔB , regressions on HDD (Figure 8A) or MAX (Figure 8B) were best fitted to quartic models that were contrasting responses relative to those observed for fraction A. With limited spontaneous heating, ΔB was positive, reaching a maximum of 3.9 percentage units; however, ΔB became negative when heating reached approximately 500 HDD or 57°C MAX, and reached a minimum of -5.0 percentage units for bales incurring the most extreme heating during storage. Coefficients of determination were greatest ($R^2 = 0.859$) for MAX, but approximately two-thirds of the variation was explained when HDD was the independent variable ($R^2 = 0.665$). For ΔC , regressions on HDD (Figure 9A) and MAX (Figure 9B) were best fitted to nonlinear models in which the independent variable was squared. Regression curves became asymptotic at -5.0 and -5.1 percentage units, respectively, indicating that the percentage of NDF that was unavailable in the rumen decreased as a function of spontaneous heating. Both regression models explained majority percentages of the variation in the data ($R^2 = 0.606$ and 0.629 , respectively).

Taken at face value, the declining nature of these response curves for ΔC suggests that the potential extent of ruminal NDF disappearance is improved by spontaneous heating; clearly, this response is inconsistent with the declining concentrations of IVTD and effective rumen degradability of DM described previously for these hays (Coblentz and Hoffman, 2009). However, a second factor required for proper interpretation of

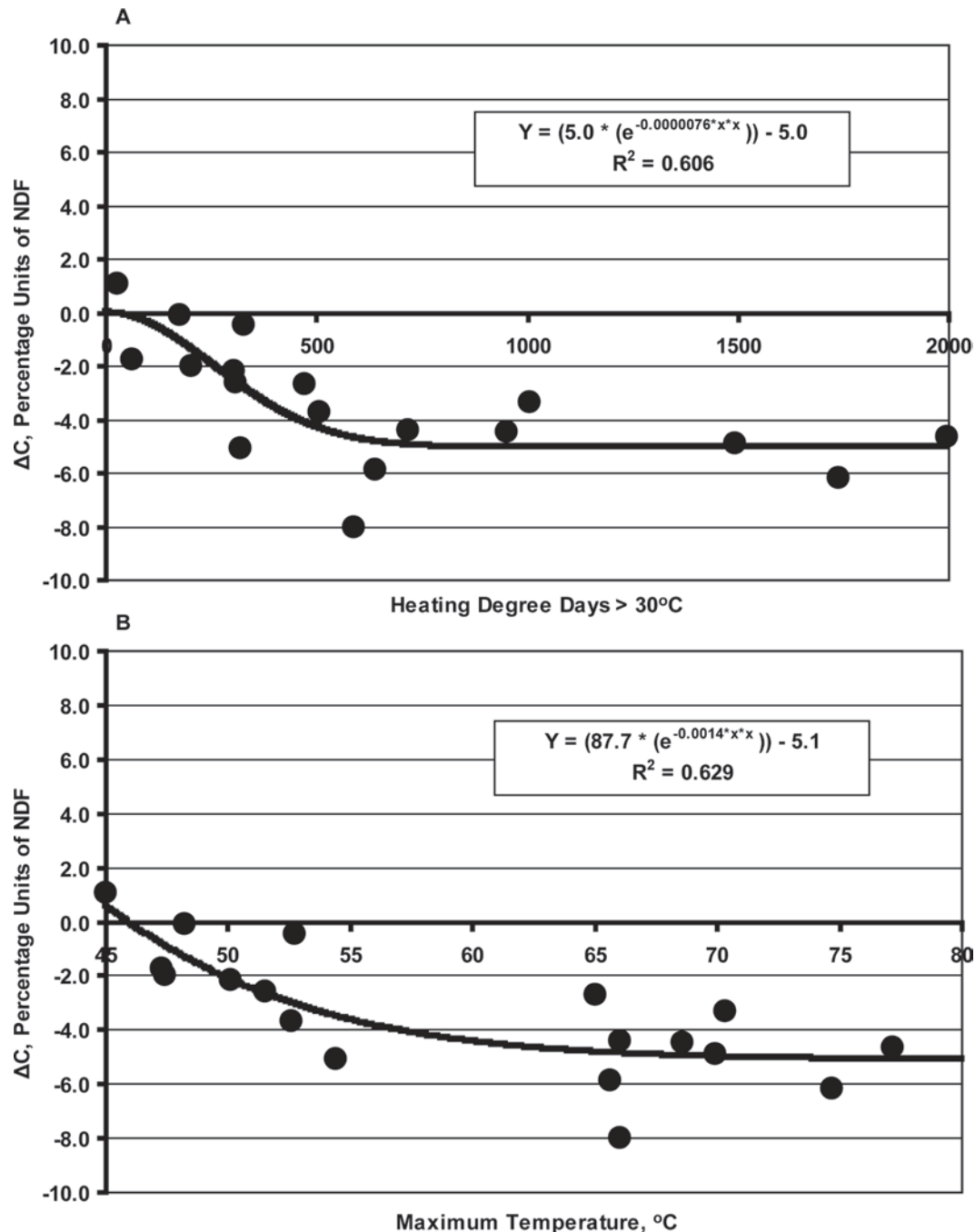


Figure 9. Changes in percentages of NDF unavailable in the rumen (poststorage – prestorage; ΔC) as affected by heating degree days >30°C (A) and maximum internal bale temperature (B). The mean prestorage concentration of fraction C (weighted based on the number of treatments from the high- and intermediate-moisture harvests) was 37.5% of NDF, which corresponds generally to $\Delta C = 0$ on the y-axis.

ruminal fiber disappearance is the actual size of the NDF pool, which became asymptotic at +8.6 percentage units in the nonlinear regression of ΔNDF on HDD (Figure 1A). Recalculation of ΔA , ΔB , and ΔC on a percentage of DM basis (ΔA_{DM} , ΔB_{DM} , and ΔC_{DM} , respectively) reflects the distribution of NDF among various ruminal disappearance pools and the total con-

centration of NDF within the hay, thereby yielding a clearer picture of changes in the actual pool sizes of fractions A, B, and C during storage. These concepts are summarized in Figure 10, where ΔA_{DM} and ΔC_{DM} are regressed on HDD. Within this context, the linear slope relating ΔC_{DM} and HDD was not significant ($P = 0.295$), and the overall mean value of ΔC_{DM} was only

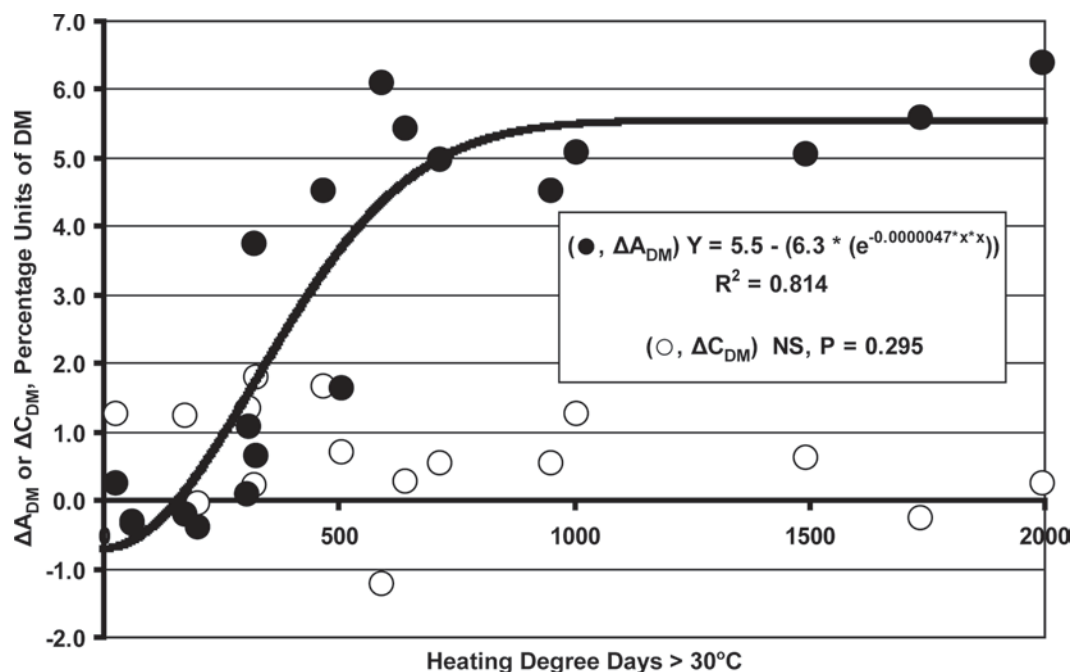


Figure 10. Changes in concentrations of fractions A and C (poststorage – prestorage) recalculated as a percentage of total forage DM (ΔA_{DM} and ΔC_{DM} , respectively) as affected by heating degree days $>30^{\circ}\text{C}$. For clarity, changes in fraction B calculated on the same basis (ΔB_{DM}) has been omitted, but data oscillated between 0.4 and 4.9 percentage units of DM (overall mean = 3.2 ± 1.3 percentage units), and was best fit with a quartic regression model ($P = 0.004$; $R^2 = 0.672$). The generally positive values for ΔA_{DM} and ΔB_{DM} indicate that the pool size of degradable fiber increased with spontaneous heating in hay. Linear regression of ΔC_{DM} on heating degree days $>30^{\circ}\text{C}$ was not significant (NS; $P = 0.295$); the overall mean for ΔC_{DM} was 0.6 ± 0.8 percentage units of DM, thereby indicating that the pool of NDF undegradable in the rumen was essentially unaffected by spontaneous heating in hay.

slightly positive (0.6 ± 0.8 percentage units of DM), indicating that the pool of rumen undegradable NDF was essentially unaffected by spontaneous heating. In addition, this suggests that the declining estimates of ΔC observed in Figure 9 occurred via simple dilution as the concentration of NDF increased within the hay because of spontaneous heating. In contrast, ΔA_{DM} and ΔB_{DM} both were consistently positive; ΔA_{DM} was best fitted to a nonlinear model that became asymptotic at 5.5 percentage units of DM (Figure 10). Estimates of ΔB_{DM} oscillated between 0.4 and 4.9 percentage units of DM that were best fitted to a quartic regression model ($P = 0.004$; $R^2 = 0.672$; data not shown). Taken in total, these data suggest that the pool of rumen degradable NDF can expand by as much as 10 percentage units of forage DM with spontaneous heating; however, it remains unclear by what combination of physical and chemical mechanisms this occurs.

Lag Time. The overall mean ΔLAG for 18 heated hays was 0.53 h, implying that on average, lag time was increased by some aspect of the storage process. However, regressions of ΔLAG on both HDD (Figure 11A) and MAX (Figure 11B) were not significant ($P \geq 0.134$) for linear, quadratic, cubic, or quartic models,

indicating there was no direct relationship between ΔLAG and spontaneous heating.

Disappearance Rate. Prestorage estimates of K_d for ruminal NDF disappearance were 0.104 and 0.094/h for HM and IM harvests, respectively, which are similar to previous reports for alfalfa (0.07 to 0.11/h, Hoffman et al., 1993; 0.075/h, Coblenz et al., 1998; 0.107/h, Ogden et al., 2005). Regressions of ΔK_d on HDD (Figure 12A) and MAX (Figure 12B) were best fitted by nonlinear models in which the independent variable was squared; in each case, ΔK_d declined with spontaneous heating until becoming asymptotic at -0.039 and $-0.043/\text{h}$, respectively. This represents a rate reduction of approximately 40% relative to unheated prestorage controls. For regressions on both HDD and MAX, R^2 statistics were quite high (0.907 and 0.883, respectively), indicating that measures of spontaneous heating were excellent predictors of K_d for ruminal NDF disappearance. Generally, these results support past work. McBeth et al. (2003) reported that K_d for NDF disappearance declined by 23% for heated bermudagrass hays, but Turner et al. (2004) reported no change in ruminal rates of NDF disappearance for heated tall fescue hays that reached a MAX of 50°C .

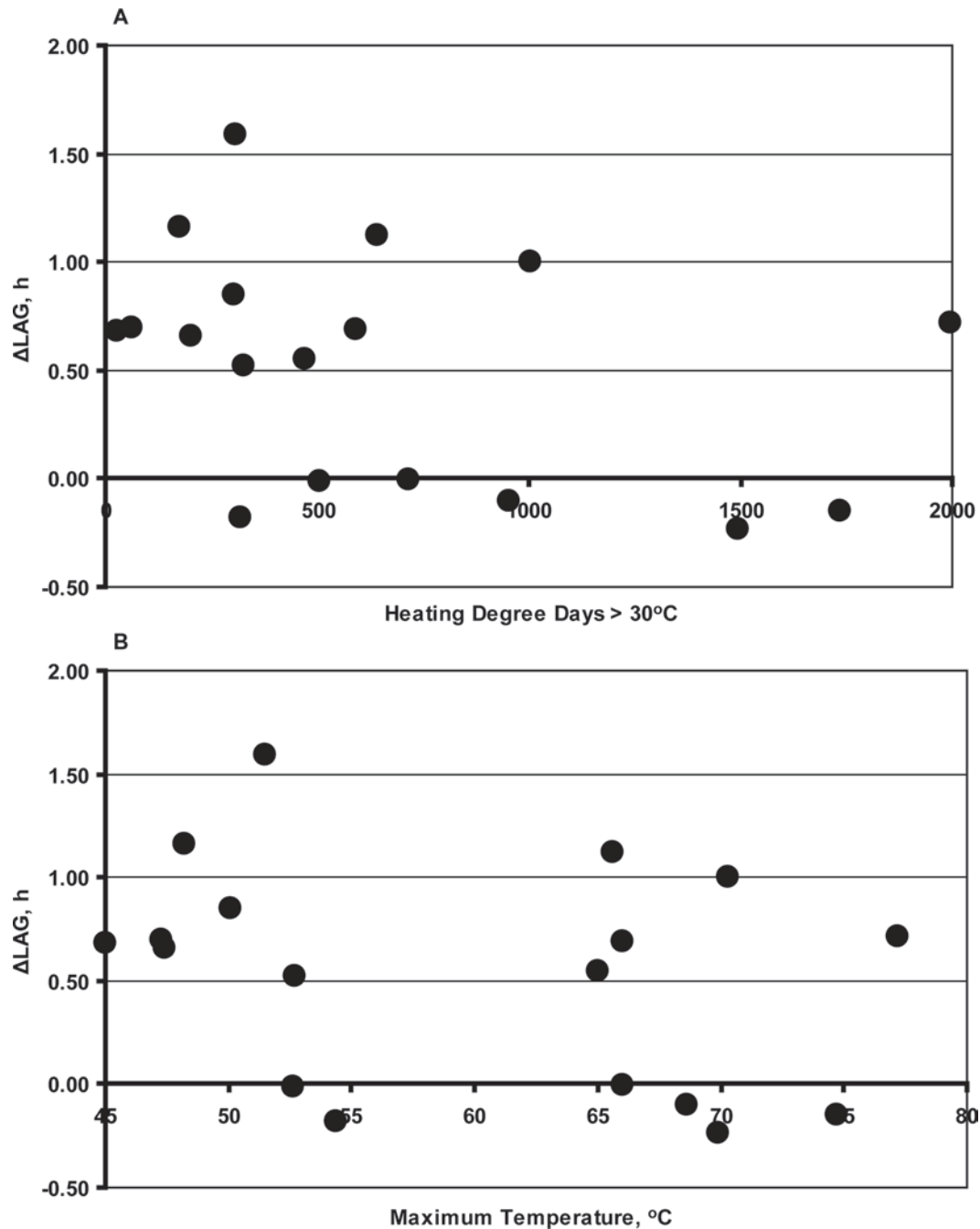


Figure 11. Changes in lag time (poststorage – prestorage; ΔLAG) as affected by heating degree days >30°C (A) and maximum internal bale temperature (B). The mean prestorage lag time (weighted based on the number of treatments from the high- and intermediate-moisture harvests) was 1.59 h, which corresponds generally to $\Delta\text{LAG} = 0$ on the y-axis. There was no relationship between ΔLAG and either measure of spontaneous heating when data were fit to quartic, cubic, quadratic, or linear regression models ($P \geq 0.134$).

Effective Degradability. Averaged over the 18 heated hays, ΔDEG ranged tightly from -1.7 to 2.8 percentage units of NDF with an overall mean change during storage of 0.2 percentage units. Linear, quadratic, cubic, and quartic regressions of ΔDEG on HDD (Figure 13A; $P \geq 0.245$) and MAX (Figure 13B; $P \geq 0.085$) were not significant, indicating there was no statistical

relationship between ΔDEG and spontaneous heating. There was a weak tendency for a positive linear relationship with MAX ($Y = 0.05x - 2.8$; $P = 0.085$; $r^2 = 0.174$), but other higher-ordered curvilinear models did not approach significance ($P \geq 0.238$). Generally, the nonexistent relationship between ΔDEG and measures of spontaneous heating is consistent with our previous

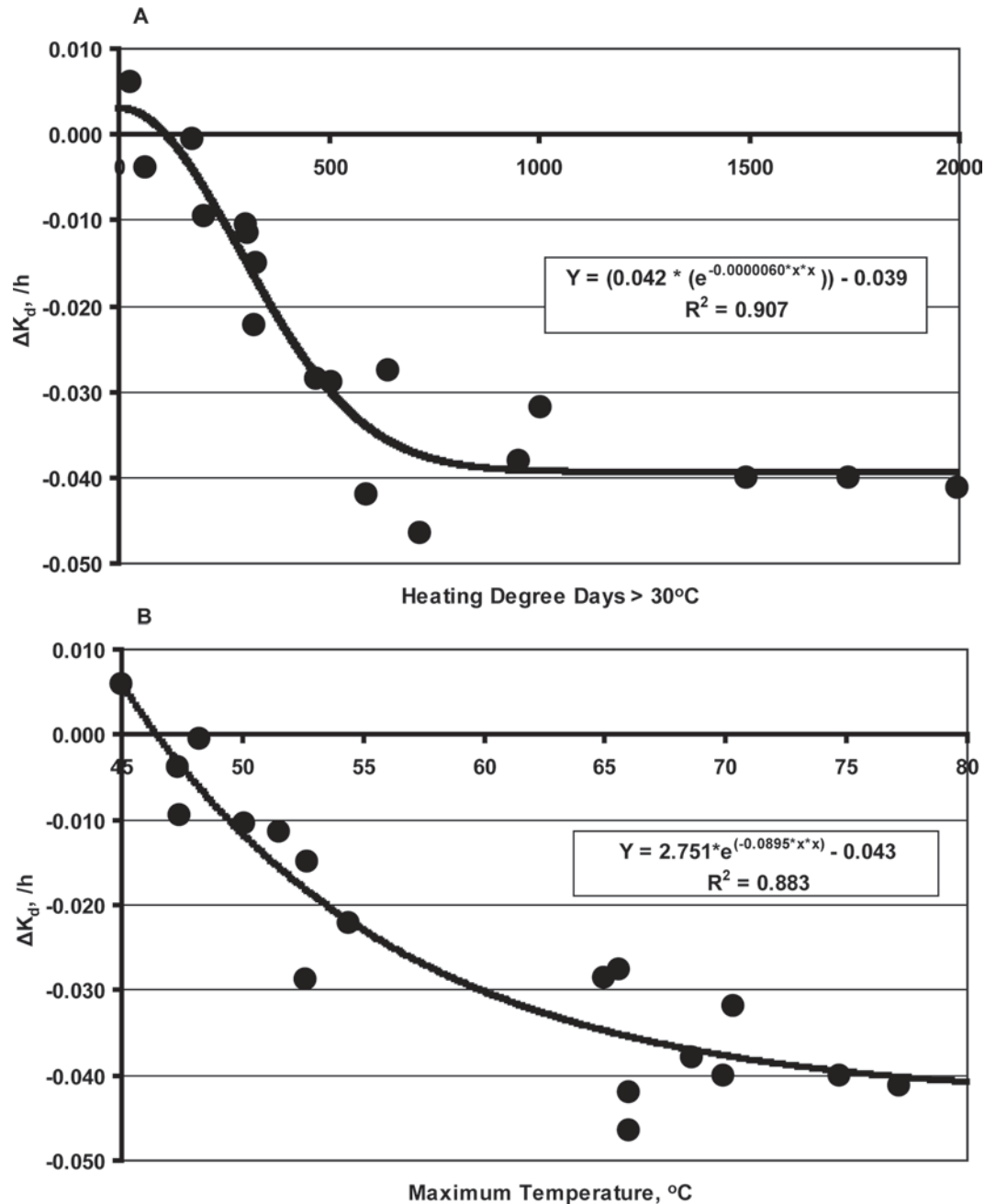


Figure 12. Changes for in situ disappearance rates of NDF (poststorage – prestorage; ΔK_d) as affected by heating degree days >30°C (A) and maximum internal bale temperature (B). The mean prestorage K_d (weighted based on the number of treatments selected from the high- and intermediate-moisture harvests) was 0.100/h, which corresponds generally to $\Delta K_d = 0$ on the y-axis.

observations for regressions of $\Delta NDFD$ on HDD (Figure 6A) and MAX (Figure 6B). In those regressions, $\Delta NDFD$ for individual hays varied erratically around $\Delta NDFD = 0$ for all but 4 hays that generally incurred the most extreme spontaneous heating.

CONCLUSIONS

The absence of any relationship between the effective ruminal degradability of NDF and spontaneous heating

is not easy to explain. The close relationships between ΔK_d and measures of spontaneous heating indicate clearly that ruminal NDF disappearance was altered negatively by some direct or indirect aspect of heating. However, it was equally apparent that these effects were offset by increases in concentrations of NDF that expanded pools of potentially degradable NDF. Although NDF is not theoretically soluble in water, the pool size for fraction A increased substantially in a nonlinear re-

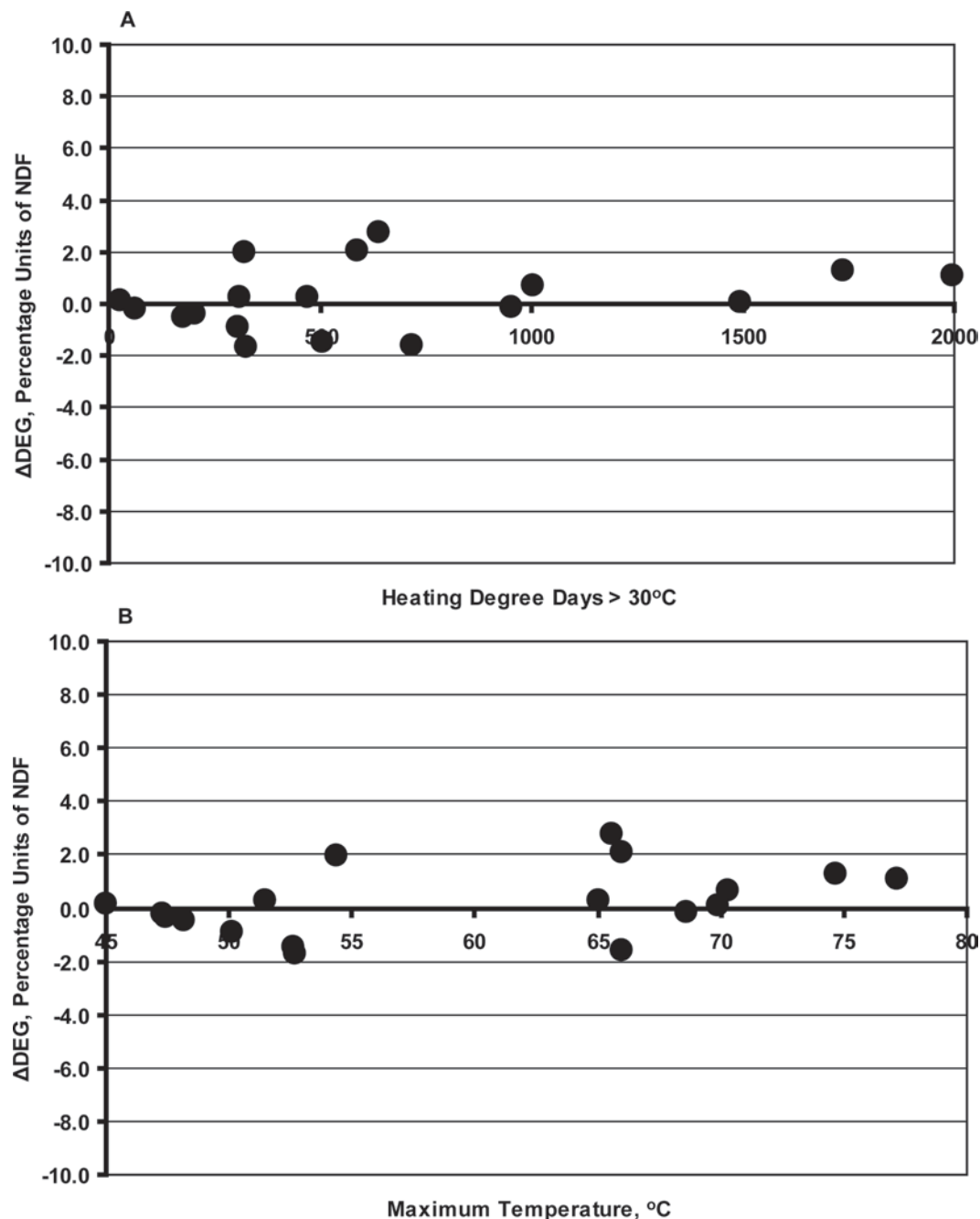


Figure 13. Changes in effective ruminal disappearance of NDF (poststorage – prestorage; Δ DEG) as affected by heating degree days $>30^{\circ}\text{C}$ (A) and maximum internal bale temperature (B). The mean prestorage effective ruminal disappearance of NDF (weighted based on the number of treatments from the high- and intermediate-moisture harvests) was 44.4% of NDF, which corresponds generally to Δ DEG = 0 on the y-axis. Effective degradability was calculated based on a 0.060/h ruminal passage rate. There was no relationship between Δ DEG and either measure of spontaneous heating when data were fit to quartic, cubic, quadratic, or linear regression models ($P \geq 0.085$).

relationship with heating, and became asymptotic at 5.5 percentage units of DM. Similarly, pool sizes for fraction B increased generally with heating, suggesting the pool of rumen degradable fiber may increase collectively by as much as 10 percentage units of DM with spontaneous heating. These results also suggest that fraction C,

which is unavailable in the rumen, was unaffected by spontaneous heating, and declining concentrations of fraction C (% of NDF basis) were largely the result of dilution by an expanding pool of potentially degradable NDF that was observed within heated hays. Regardless of the mechanisms involved, the relative insensitivity

of effective ruminal degradability of NDF or NDFD to spontaneous heating implies that associated reductions in effective ruminal DM disappearance described in our companion report (Coblentz and Hoffman, 2009) occurred primarily because of declining concentrations of cell solubles, and not via reductions in digestible forage fiber. Similarly, reduced energy densities within heated hays also are likely to be related primarily to a shrinking pool of cell solubles, especially when the NDFD option is used to estimate truly digestible fiber within the summative model (NRC, 2001).

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