Effects of Nighttime Air Temperature During Kernel Development of Field-Grown Rice on Physicochemical and Functional Properties

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ABSTRACT

Elevated nighttime air temperatures (NTATs) occurring during critical grain-filling stages affected rice physicochemical properties, which impacted functional quality. Six cultivars were grown at multiple field locations from northern to southern Arkansas during 2007 to 2010. Nighttime temperatures were recorded throughout production at each of the locations, and 95th percentiles of NTATs were calculated for each cultivar's reproductive (R) stages. Amylose content and crude protein content decreased linearly, whereas total lipid content increased linearly, with increasing NTATs occurring during the grain-filling stages (R6–R8). Effects of NTAT on proximate composition influenced functional properties. Peak viscosities increased linearly as NTAT increased, whereas setback viscosities decreased. Setback viscosities were linearly correlated to NTATs for medium-grain cultivars, but correlations were quadratic for the long-grain cultivars. Gelatinization temperatures increased linearly with increasing NTAT. The R stages in which correlations were strongest varied by cultivar and by property, hypothesized to result from differences in kernel development patterns among cultivars. These findings have significant implications for rice production scientists and processors, in that understanding the effects of NTAT on physicochemical and functional properties may help explain and reduce quality variation.

Recent studies in both controlled-temperature and field-scale environments have established that elevated nighttime air temperatures (NTATs) occurring during critical grain-filling stages affect rice kernel development, resulting in reduced yield, increased kernel chalkiness, and reduced milling quality (Peng et al 2004; Cooper et al 2006, 2008; Ambardekar et al 2011; Lanning et al 2011). Other studies have shown that the chemical makeup of starch is affected by elevated NTAT, as evidenced by decreased amylose content (AC) and changes in ratios of long- to short-chain amylopectin (Counce et al 2005; Cooper et al 2008). Several hypotheses have been presented to explain the effects of NTAT stress, including reduced substrate supply to the endosperm, initiating slow starch granule growth and irregular granular organization (Fitzgerald and Resurreccion 2009) and disruption of enzymatic activity responsible for starch formation (Counce et al 2005). Although the underlying mechanisms that tie the effects of NTAT to the structural and functional changes of starch are not clearly established, these findings are critical to rice end-use applications, because functional properties of milled rice, which directly impact cooking and sensory quality, are primarily determined by starch physicochemical properties.

AC of a given rice strain is determined by the expression of two alleles of a polymorphic waxy gene, Wx^a and Wx^b, which regulate amylose synthesis by controlling the activities of grain-bound starch synthase (GBSS) enzymes (Counce et al 2005). Suzuki et al (2003) reported that mutations to Wx^a and Wx^b alleles could reduce amylose accumulation in the starchy endosperm, thus affecting the end-use quality of rice. The study further reported that in addition to the genetic effect, elevated temperatures during grain-filling stages disrupt enzyme pathways that determine amylose accumulation. Jiang et al (2003) investigated the enzymes responsible for biosynthesis of amylose and amylopectin in a nonwaxy indica rice cultivar as a function of high NTAT during the kernel-maturation stage, which was simulated in phytotrons. The study reported that GBSS activity was less at a simulated nighttime temperature of 35°C than at 28°C. Correspondingly, AC and branching frequency of amylopectin were reduced at 35°C. Other studies have reported that high NTATs during rice grain-filling stages impede metabolic pathways and inhibit starch deposition, resulting in reduced AC, decreased amylopectin branching, increased chalk, and decreased kernel weight (Yamakawa et al 2007; Yamakawa and Hakata 2010).

Protein and lipids are also known to have an effect on cooked rice characteristics (Hamaker and Griffin 1993; Han and Hamaker 2001; Fitzgerald and Resurreccion 2009). Hamaker and Griffin (1993) reported that specific proteins linked to amylose by disulfide bonds in a starch granule are known to influence cooked rice texture and sensory attributes. A greenhouse study by Tamaki et al (1989) evaluated the effect of simulated night temperatures of 14, 20, and 26°C on amino acid content of high- and low-protein cultivars. Results indicated that regardless of the protein content, cultivars exposed to 26°C showed a reduction in free amino acids in comparison to those grown at lower temperatures. A study by Fitzgerald and Resurreccion (2009) indicated that the proportion of protein relative to starch increased with elevated temperatures but that the total amount of protein was not affected.

Lipid bodies in rice are found predominantly in the aleurone layer of the bran (Lloyd et al 2000) but are also present in the endosperm, cross-linked with starch (Choudhury and Juliano 1980). These lipids, present in milled rice, are understood to influence the viscoelastic properties of starch by forming inclusion complexes with the helical structures of amylose, which affects the swelling ability of starch granules (Liang et al 2002). Cooper et al (2008) found total lipid content (TLC) of some cultivars to increase at a NTAT of 30°C, compared with 18, 22, and 26°C.

Although the studies mentioned have established that elevated NTATs affect proximate components of rice, there is relatively limited research demonstrating that functional properties of rice are influenced by NTAT. Gelatinization, referring to the melting of the crystalline structure of starch, must occur before swelling of the starch granule begins; therefore, gelatinization temperature (GT) is strongly correlated to cooking duration and texture of cooked rice (Maningat and Juliano 1980). This process is generally thought to be governed by the amylopectin structures present in a given sample (Umemoto et al 2002; Cameron et al 2008) and occurs over a range of temperatures, typically discussed in terms of onset, peak, and conclusion temperatures. Aboubacar et al (2006) reported lower ACs, as well as lower proportions of short-chain amylopectin, in nine long-grain cultivars grown in Beaumont, TX, where the average night temperature during the grain-filling period was reported to be 24°C, compared with rice grown in Glennonville, MO, where NTATs averaged 19°C. The greater-

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temperature growth environments also resulted in greater onset, peak, and conclusion GTs.

The pasting profile of a given starch sample provides information regarding its processing quality. When starch is dispersed in an aqueous solution and heated, the starch granules absorb water, resulting in increased viscosity. Peak viscosity (PV) is reached when the number of swollen but intact granules is at its maximum, and it is therefore an indicator of water-binding capacity (Dang and Copeland 2004). With the application of shear force, the granules are disrupted, and straight-chain amylose molecules leach out, resulting in a breakdown in viscosity, or thinning of the paste. As the starch solution cools, the molecules reassociate in an ordered structure, such that a gel is formed. The strength of the gel is dependent on several factors, including the cooling rate, amylose/amylopectin ratio, and other molecules, such as protein, present in the system (Hamaker and Griffin 1990). The difference between the final viscosity (FV) and the PV is referred to as setback viscosity (SV), and it is often considered indicative of a starch's processing quality (Bergman et al 2004).

A glasshouse study by Lisle et al (2000) compared the pasting properties of three rice cultivars grown at NTATs of 15 and 21°C. Results indicated that pasting temperatures and PVs were greater in the samples grown at 21°C than in those grown at 15°C. In a three-year field study of three indica cultivars, Dang and Copeland (2004) reported that the growing season with the highest average minimum (i.e., nighttime) temperature yielded the greatest PVs and the lowest FVs. In contrast, with the exception of Cypress, results of a phytotron study conducted by Cooper et al (2008) indicated no significant changes in PVs of rice cultivars grown at a NTAT of 30°C, compared with those grown at NTATs of 18, 22, and 26°C.

The majority of research investigating the effects of NTAT has been conducted under highly controlled conditions or based on historical data. The current study aimed to provide a comprehensive and systematic design that not only accounted for the natural variation in environmental temperature occurring across years and locations but also evaluated its impact on a field-scale level. The objective of the study was to quantify the effects of elevated NTATs occurring during specific grain-filling stages on kernel physicochemical components and end-use functional properties.

MATERIALS AND METHODS

Sample Production

Data were obtained during 2007, 2008, 2009, and 2010 from the Arkansas Rice Performance Trials, a program comprising multiple planting locations throughout Arkansas, with replicated plots of multiple cultivars at each location. Three pure-line long-grain (Cypress, LaGrue, and Wells), one hybrid long-grain (XL723), and two pure-line medium-grain (Bengal and Jupiter) cultivars were grown at each of the following Arkansas locations: Corning, Newport, Stuttgart, and Rohwer in 2007; Corning, Pine Tree, Stuttgart, and Rohwer in 2008; Keiser, Pine Tree, Stuttgart, and Rohwer in 2009; and Keiser, Newport, Pine Tree, Stuttgart, and Rohwer in 2010. Growing locations were strategically selected to span from a northern (36.4°N) to southern latitude (33.8°N) in an effort to increase the probability of NTAT differences during rice reproductive stages.

In a randomized block design, 18 experimental plots were assigned at each location, such that each of the six cultivars was planted in three randomly assigned plots. Each of the five pure-line cultivars was drill-seeded at a rate of 428 seeds/m^2 in a nine-row (0.2 m spacing) plot, 4.6 m in length. The hybrid cultivar was sown in plots of the same dimensions at a rate of 171 seeds/m^2. Management practices included planting dates, flooding, fertilization (nitrogen rates varied depending on conditions, from 120 to 165 kg/Ha), and pesticide applications to achieve near-optimum yields.

Staging and Thermal Unit Accumulation

Each year of the study, ambient temperatures were recorded in 30 min increments by two temperature and relative humidity sensors (HOBO Pro/Temp Data Logger, Onset Computer, Bourne, MA) positioned at each growing location. Cultivars grown in Stuttgart, Arkansas, were visually observed throughout their reproductive developmental (R) stages (Counce et al 2000). The day of year (DOY) upon which the distinguishing morphological characteristics of each R stage (R2–R9) appeared was recorded for each cultivar as the average of seven plants in each of three replicate plots. At the other locations, only the R3 stage, commonly referred to as the "50% heading date," or the DOY upon which 50% of the panicles in a plot were judged to have emerged, was visually identified in triplicate and recorded as an average for each cultivar. The subsequent stages and the DOYs of their initiation for each cultivar at each location were estimated from the Stuttgart staging progression data, along with the amounts of thermal energy accrued through air temperature exposure at each location, as described subsequently.

Accumulation of daily thermal energy units, quantified as DD10 units, for each reproductive growth stage, beginning with the initiation of R3 to the end of R8, was calculated by the method of Ambardekar et al (2011) from the following equation:

$$\mathrm{DD10} = \sum_{i=1}^{48}\left[\left(\frac{T_{\mathrm{MAX}}\ (^{\circ}\mathrm{C}) + T_{\mathrm{MIN}}\ (^{\circ}\mathrm{C})}{2}\right) - 10^{\circ}\mathrm{C}\right] \times 0.5\ \mathrm{hr} \times \frac{1\ \mathrm{day}}{24\ \mathrm{hr}}$$

where T_{MAX} and T_{MIN} represented the maximum and minimum temperatures, respectively, during a 30 min interval. Maximum temperature was considered 34°C if the maximum temperature during a 30 min interval was greater than 34°C. Growth under 10°C was assumed negligible, hence, not considered in this study.

Ambient temperatures during the time of the day extending from 8:00 p.m. to 6:00 a.m. were considered as NTATs. Frequencies of the NTATs were tallied within each cultivar's respective R stages for each year and location. The 95th percentiles of NTAT frequency (NT_{95}), that is, the temperatures below which 95% of the NTATs occurred, were calculated following a cumulative frequency distribution model (JMP version 8.2, SAS Institute, Cary, NC).

Previous studies, which related NT_{95} occurrence during the R3–R8 stages to increased kernel chalkiness and decreased head rice yield, reported strong correlations during the R6, R7, and R8 stages (Ambardekar et al 2011; Lanning et al 2011). It is during the R6–R8 stages, also referred to as the grain-filling stages, that glucose is converted into starch and starch accumulates in the endosperm. These physiological processes are known to be sensitive to elevated NTATs (Counce et al 2005; Cooper et al 2008). Hence, NTATs occurring in the R6, R7, and R8 stages and their effects on functional properties were the primary focus of this study.

Sample Collection

In each of the study years and locations, samples from each of the 18 cultivar-replication plots were harvested over a range of moisture contents (MCs); MCs are expressed on a wet basis, unless otherwise specified. The harvest MCs across all plots ranged from 11.4 to 28.6% in 2007, 12.7 to 26.9% in 2008, 13.0 to 28.9% in 2009, and 15.8 to 25.5% in 2010. Each harvested sample comprised approximately 120 randomly hand-cut panicles. Kernels from five panicles were used to determine field MC with a single-kernel moisture meter (CTR800E, Shizuoka Seiki Co., Fukurui City, Shizuoka, Japan). Kernels from the remaining panicles, yielding approximately 600 g, were stripped with a portable mechanical thresher (SBT, Almaco, Nevada, IA) and stored in cotton sample bags until sample preparation.

Sample Preparation

Harvested rice samples were cleaned (dockage tester, Carter-Day, Minneapolis, MN) and dried in a temperature- and humidity-controlled chamber (AA5582, Parameter Generation & Control, Black Mountain, NC) maintained at 26°C and 56% relative humidity, corresponding to a rough rice equilibrium MC of approximately 11.5% (ASABE 2009). Dried rough rice MCs ranged from 12.0 to 12.8%, determined with a convection oven (1370-FM, Sheldon Manufacturing, Cornelius, OR) in which duplicate 15 g samples were dried according to the method of Jindal and Siebenmorgen (1987). After drying, samples were stored in resealable plastic bags at 4°C, pending further analysis.

The number of rice lots analyzed for proximate and functional attributes varied according to prevailing field drying conditions and targeted harvest MCs during a given season. Table I provides a summary of the total lots analyzed for TLC, crude protein content (CPC), pasting properties, AC, and GT throughout the four-year study.

TLC

TLC was measured on samples harvested during 2007, 2009, and 2010 (Table I). In 2007, one plot replicate from at least one cultivar–location–harvest MC (HMC) combination was analyzed; in some cases, multiple HMCs and plot replicates were analyzed, based on sample quantity and availability. In 2009, two plot replicates from each cultivar–location–HMC combination were tested. In 2010, the sampling schedule was reduced to target optimal HMCs. Thus, because the number of HMCs was reduced, TLC was analyzed for each of the three plot replicates for each cultivar–location–HMC combination.

Rough rice (100 g) from each sample was dehulled in a laboratory sheller (THU, Satake, Tokyo, Japan), with a clearance of 0.048 cm between the rollers, to produce brown rice, from which TLC was determined. It is to be noted that with the sheller roller clearance used, approximately 92% of the rough rice in each sample was dehulled. The remaining 8% of kernels, with hulls intact, were manually removed from the brown rice before measuring TLC.

A sample mill (3010-30, UDY, Fort Collins, CO) fitted with a 100 mesh (0.5 mm) screen was used to grind the brown rice into flour. Brown rice TLC was determined in duplicate with a lipid extraction system (Avanti 2055, Foss North America, Eden Prairie, MN) according to AACC International Approved Method 30-20.01 (AACCI 2010), with modifications to the petroleum ether washing duration, as described by Matsler and Siebenmorgen (2005). TLC was expressed as the mass percentage of extracted lipid to the original brown rice.

TABLE I
Numbers of Lots Analyzed for Total Lipid Content (TLC), Crude Protein Content (CPC), Pasting Profile (PP), Amylose Content (AC), and Onset Gelatinization Temperature (GT_{onset}) by Year, Location, and Cultivar

		Cultivars														
		Bengal					Jupiter					Cypress				
Harvest Year	Location[a]	Total Lots[b]	TLC	CPC	PP	AC and GT_{onset}	Total Lots[b]	TLC	CPC	PP	AC and GT_{onset}	Total Lots[b]	TLC	CPC	PP	AC and GT_{onset}
2007	C	18	3	na[c]	18	na	20	7	na	20	na	19	4	na	19	na
	N	19	1	na	19	na	17	3	na	17	na	19	6	na	19	na
	S	18	3	na	18	na	17	3	na	16	na	18	4	na	18	na
	R	14	1	na	14	na	9	3	na	9	na	11	3	na	11	na
2008	C	20	na	5	20	na	20	na	8	20	na	10	na	6	10	na
	P	13	na	5	13	na	12	na	4	12	na	9	na	5	9	na
	S	18	na	6	18	na	18	na	5	18	na	17	na	5	17	na
	R	7	na	2	7	na	6	na	1	6	na	9	na	4	9	na
2009	K	6	4	4	6	3	6	4	4	6	na	6	4	4	6	0
	P	12	8	8	12	3	12	8	8	12	na	12	8	8	12	3
	S	15	10	10	15	3	15	10	10	15	na	15	10	10	15	6
	R	9	6	6	9	3	9	8	6	9	na	12	8	8	12	3
2010	K	3	3	3	3	3	3	3	3	3	na	3	3	3	3	3
	N	3	3	3	3	3	3	3	3	3	na	3	3	3	3	3
	P	3	3	3	3	3	3	3	3	3	na	3	3	3	3	3
	S	3	3	3	3	3	3	3	3	3	na	3	3	3	3	3
	R	3	3	3	3	3	3	3	3	3	na	3	3	3	3	3

		Cultivars														
		LaGrue					Wells					XL723				
2007	C	15	3	na	15	na	15	5	na	15	na	15	5	na	11	na
	N	18	3	na	18	na	18	3	na	17	na	21	6	na	18	na
	S	16	8	na	16	na	17	7	na	17	na	13	4	na	13	na
	R	8	2	na	8	na	13	5	na	11	na	10	3	na	8	na
2008	C	5	na	0	5	na	19	na	7	19	na	11	na	0	11	na
	P	12	na	4	11	na	12	na	4	12	na	11	na	4	11	na
	S	14	na	6	14	na	17	na	7	17	na	17	na	6	17	na
	R	6	na	3	6	na	8	na	2	8	na	11	na	3	11	na
2009	K	6	4	4	6	0	6	4	2	6	na	6	4	4	6	2
	P	12	8	8	12	3	12	8	8	12	na	12	7	7	12	3
	S	12	8	8	12	6	12	8	8	12	na	15	10	10	15	6
	R	9	6	6	9	6	9	5	5	9	na	12	8	8	12	3
2010	K	3	3	3	3	3	3	3	3	3	na	3	3	3	3	3
	N	3	3	3	3	3	3	3	3	3	na	3	3	3	3	3
	P	3	3	3	3	3	3	3	3	3	na	3	3	3	3	3
	S	3	3	3	3	3	3	3	3	3	na	3	3	3	3	3
	R	3	3	3	3	3	3	3	3	3	na	3	3	3	3	3

[a] C = Corning; K= Keiser; N = Newport; P = Pine Tree; R = Rohwer; and S = Stuttgart (all locations in Arkansas).
[b] Total lots comprise multiple harvest moisture contents from replicate (single, duplicate, or triplicate) plots, as harvest conditions allowed.
[c] na = not analyzed.

CPC

CPCs of brown rice samples from the 2008, 2009, and 2010 harvest seasons were measured (Table I). In 2008, one plot replicate from each cultivar–location–HMC combination was tested, as sample quantity allowed; in 2009, two plot replicates from each combination were tested. In 2010, as with TLC, protein was tested on each of the triplicate plots from every cultivar–location–HMC combination.

Brown rice flour was prepared according to the procedure described for TLC. A nitrogen analyzer (FP-2000, Leco, St. Joseph, MI) was used to measure nitrogen content of each ground sample (single assay), and a factor of 6.25 was applied to estimate CPC, as described in AACCI Approved Method 46-30.01 (2010).

Pasting Properties (PV and SV)

The pasting profile for each harvested sample in each of the four study years was determined in duplicate using ground samples of head rice (Table I). To obtain head rice, rough rice samples were first removed from cold storage and allowed to equilibrate at room temperature for at least 24 hr. Duplicate 150 g rough rice subsamples from each sample were dehulled, and the resultant brown rice subsamples were milled for 30 sec with a laboratory mill (McGill No. 2, RAPSCO, Brookshire, TX). Head rice was separated from broken kernels with a sizing device (Seedburo Equipment, Chicago, IL). Twenty grams of each head rice fraction was ground into flour with the sample mill described previously, fitted with a 0.5 mm screen.

MC was measured for each flour sample by placing 2.0 g of flour in an oven at 130°C for 1 hr according to AACCI Approved Method 44-15.02 (2010). The MC was used to determine the appropriate amount of water to add to the flour to attain a 12% MC mixture (db). Approximately 3 ± 0.01 g of flour was mixed with approximately 25 ± 0.05 g of deionized water in an aluminum canister. PV and FV were determined with a viscometer (RVA Super 4, Newport Scientific, Warriewood, NSW, Australia) according to AACCI Approved Method 61-02.01 (2010). SV was calculated as FV minus PV.

AC

Apparent AC of head rice flour from lots of Bengal, Cypress, LaGrue, and XL723 harvested in 2009 and 2010 was determined (Table I). Duplicate samples of head rice flour were prepared as described for pasting properties, except that a 0.25 mm mill screen was used.

A standard curve for AC was developed with ground rice flour samples of predetermined AC, ranging from zero to 25%. Samples were ground from head rice of long-grain cultivars Dixiebelle and Lemont, medium-grain cultivar Bengal, and waxy cultivar Calmochi, obtained from the USDA ARS Dale Bumpers National Rice Research Center, Stuttgart, Arkansas. The samples were refrigerated before analysis and equilibrated at 25°C for 2 hr before standard curve preparation. Apparent AC was determined by the method of Williams et al (1958), adapted for use with an automatic analyzer (AutoAnalyzer 3, Seal Analytical, Mequon, WI) using a wavelength of 620 nm (Juliano 1971). The resulting average absorbance values were plotted against the known amylose percentages to generate a standard curve. Experimental samples were analyzed according to the procedure outlined previously, and the AC of each sample was estimated from the equation associated with the standard curve.

GT

The head rice flour samples prepared for AC measurement were also used to measure GT with a differential scanning calorimeter (DSC) (Pyris-1, Perkin Elmer, Norwalk, CT). Duplicate 4 mg (dry matter equivalent) flour samples were hydrated for 24 hr with 8 µL of deionized water in hermetically sealed aluminum DSC pans before analysis. The DSC was programmed to heat each sample from 25 to 120°C at a rate of 10°C/min. An empty DSC pan was used as a reference. Onset and peak GTs (GT_{onset} and GT_{peak}) were determined from DSC thermograms generated by the instrument's software (Pyris series, version 9.1, Perkin Elmer).

Statistics

Coefficients of determination (R^2) and their significance were determined by analysis of variance at $\alpha = 0.05$ with polynomial regression analysis (JMP version 8.2). Correlation coefficients (r values) between AC, CPC, TLC, PV, SV, and GT, and corresponding NT_{95} during an R stage for each year–cultivar–location combination were determined with multivariate analysis (JMP version 8.2).

RESULTS AND DISCUSSION

NTAT Effects on Proximate Components

Temperature data collected throughout the four-year study show that years 2007 and 2010 generally exhibited greater NTATs

TABLE II
Average Nighttime Air Temperatures (NTATs) Recorded Throughout the R6–R8 Stages of Rice Reproductive Development During Harvest Years 2007–2010 for Each Cultivar at Each Growing Location

		Average NTAT[a] (°C)							
Year	Location	Bengal	Jupiter	Cypress	LaGrue	Wells	XL723	Across Cultivars	Across Cultivars and Locations
2007	Corning	22.7	22.9	24.6	21.8	21.7	21.8	22.6	24.4
	Newport	21.1	21.4	21.4	21.4	21.1	22.4	21.5	
	Stuttgart	29.3	29.2	29.4	29.4	29.5	29.1	29.3	
	Rohwer	24.4	24.4	24.5	24.5	23.8	22.4	24.0	
2008	Corning	21.4	22.3	20.0	22.3	24.2	23.8	22.3	22.7
	Pine Tree	21.4	21.3	21.4	21.2	21.3	21.3	21.3	
	Stuttgart	24.2	24.1	24.6	24.4	24.4	23.7	24.2	
	Rohwer	22.5	24.8	23.1	22.8	22.5	22.1	23.0	
2009	Keiser	21.3	21.4	21.4	19.6	20.5	21.3	20.9	21.3
	Pine Tree	18.9	19.1	17.6	18.1	19.3	19.8	18.8	
	Stuttgart	26.5	25.9	25.5	25.4	25.4	26.6	25.9	
	Rohwer	19.2	21.3	19.2	20.0	19.4	18.9	19.7	
2010	Keiser	25.9	26.4	25.9	24.6	25.7	26.3	25.8	25.9
	Newport	20.9	21.1	20.9	20.3	20.9	20.7	20.8	
	Pine Tree	26.6	25.6	26.0	26.3	26.1	25.8	26.1	
	Stuttgart	30.3	29.3	29.6	29.9	29.8	29.6	29.8	
	Rohwer	27.3	27.6	27.2	26.8	27.2	27.3	27.2	

[a] Average ambient air temperatures recorded at 30 min intervals during the time of day extending from 8:00 p.m. to 6:00 a.m.

than 2008 and 2009. Table II provides the mean NTATs recorded during reproductive stages R6–R8 in each of the four years of the study for each cultivar and location. Yearly fluctuations were especially notable in the southernmost locations of Stuttgart and Rohwer. These annual differences are reflected in the calculation of NT_{95}, the temperature value below which 95% of all NTATs fell for a given year–location–cultivar–R stage. This NT_{95} value was determined as a means of providing one temperature value with which to correlate functional and physicochemical property values that were observed for each year–location–cultivar combination.

Table III shows correlation coefficients calculated to establish the relationships between NT_{95} and AC, CPC, and TLC occurring during the R6, R7, and R8 stages for all of the cultivars grown from 2007 to 2010. For each of the proximate components, the R stages during which the strongest correlations with NT_{95} were observed varied among cultivars. Although it is well documented that sucrose is deposited in the endosperm and converted to starch through a series of enzymatic steps during the R6 through R8 stages (Avigad and Dey 1996; Counce et al 2005), elevated NTATs during any one or all of these critical grain-filling stages could disrupt the starch-packing and sucrose-conversion processes. Differences in R stage–specific correlations within cultivars indicate nonhomogeneous development of kernels on a panicle from one cultivar to another. By definition, the R6 stage represents the period during which metabolic processes responsible for starch accumulation and actual endosperm formation occur (Counce et al 2005). Ambardekar et al (2011), who observed increasingly strong correlations of chalk formation with NT_{95} from the R6–R8 stages, reported that a plant classified in the R8 stage will exhibit a large number of kernels still in the R6 or R7 stages and thereby reasoned that NTATs were actually affecting the metabolic processes occurring in these early grain-filling stages. This discrepancy between plant stage identification and the distribution of kernel stages within the plant inherently results from the manner in which the Counce staging system is applied, wherein staging is based on the first kernel progression on the main-stem tiller (Counce et al 2000).

Counce and Gravois (2006) reported that sucrose synthase enzyme activity during the critical grain-filling stages was directly proportional to culm and panicle densities; however, specific activity of sucrose synthase was less in the top half of panicles than in the bottom half. Thus, disparities observed in the correlations of proximate properties versus NT_{95} could also be a function of the location of developing kernels on a panicle and the degree of exposure to NTAT during a given R stage.

AC

Overall, negative correlations of AC with NT_{95} were highly significant across all tested cultivars and R stages (Table III). Figure 1 illustrates decreasing AC with increasing NT_{95} during the R8 stage. Although not significantly different, the trends in slopes of Bengal (–0.82) and Cypress (–0.60) compared with LaGrue (–0.42) and XL723 (–0.44) indicate faster declines in AC with each unit of temperature increase. A phytotron study by Cooper et al (2008) showed that increased NTATs resulted in decreased AC of Bengal, Cypress, and LaGrue; however, no differences in AC were observed in XL8, XP710, and M204. These findings concur with the current study and suggest that cultivars vary in susceptibility to NTAT impact on AC reduction. The degree of temperature susceptibility among cultivars appears to be influenced by the occurrence of a single-nucleotide polymorphism in the DNA sequence (Larkin and Park 1999). This same genetic mutation has been shown to be correlated with cultivars of lesser AC (<18%) (Ayres et al 1997).

CPC

Negative correlations between CPC and NT_{95}, indicating decreasing protein with increasing NTAT, were generally observed for all cultivars. The overall strength of correlation between CPC and NT_{95}, as indicated by the correlation coefficients in Table III, was less than that observed for AC. Long-grain cultivar Cypress did not exhibit significant correlation in any R stage, suggesting that it is least susceptible to the effects of NTAT on protein. Bengal, Jupiter, LaGrue, and Wells each showed increasing strength of correlation from R7 to R8 but did not show significant correlation in R6. Hybrid cultivar XL723 was the only cultivar to show greater correlation in the R6 stage than in R7 or R8. Figure 2 illustrates the response of CPC to increased NT_{95} during the R8 stage. Bengal and XL723 exhibited the steepest negative slopes (–0.25 for both), suggesting that these cultivars were most sensitive to NTAT effects on protein content.

These findings contradict those of Fitzgerald and Resurreccion (2009), who observed an increased proportion of proteins relative to total kernel mass owing to elevated temperature exposure. They hypothesized that kernel mass decreased as a result of less starch accumulation. A study by Lin et al (2010) found

TABLE III
Correlation Coefficients of Amylose, Crude Protein, and Total Lipid Contents, Peak and Setback Viscosities, and Onset Gelatinization Temperature (GT_{onset}) with the 95th Percentiles of Nighttime Air Temperature Frequencies During the R6, R7, and R8 Reproductive Stages of Medium- and Long-Grain Rice Cultivars[a]

		Medium-Grain Cultivars		Long-Grain Cultivars			
Property	**R Stage**	**Bengal**	**Jupiter**	**Cypress**	**LaGrue**	**Wells**	**XL723**
Amylose content	R6	–0.91	na	–0.84	–0.94	na	–0.86
	R7	–0.94	na	–0.86	–0.84	na	–0.90
	R8	–0.91	na	–0.96	–0.86	na	–0.85
Crude protein content	R6	ns	ns	ns	ns	ns	–0.73
	R7	–0.62	–0.58	ns	ns	ns	–0.59
	R8	–0.63	–0.68	ns	–0.77	–0.64	–0.65
Total lipid content	R6	0.92	0.72	0.86	0.82	0.71	0.68
	R7	0.89	0.85	0.81	0.78	0.62	0.81
	R8	0.73	0.88	0.78	0.77	0.90	0.84
Peak viscosity	R6	ns	0.50	0.81	0.72	0.59	0.69
	R7	0.57	0.60	0.79	0.82	0.65	0.68
	R8	ns	0.61	0.78	0.73	0.66	0.74
Setback viscosity	R6	–0.67	–0.57	–0.88	–0.79	–0.71	–0.66
	R7	–0.82	–0.80	–0.76	–0.85	–0.69	–0.68
	R8	–0.64	–0.77	–0.75	–0.83	–0.67	–0.66
GT_{onset}	R6	0.86	na	0.82	0.96	na	0.92
	R7	0.93	na	0.80	0.85	na	0.83
	R8	0.79	na	0.95	0.92	na	0.86

[a] Rice was grown in Arkansas from 2007 to 2010; na = not available, and ns = not significant ($P > 0.05$).

no significant difference in CPC resulting from NTAT but did report that prolamin and globulin components decreased in response to increasing NTAT. Thus, differences in cultivar susceptibility may be owing to varying ratios and sensitivities of different protein constituents.

TLC

The effects of NTAT on TLC were positive and significant for all cultivars and tested R stages (Table III), indicating that TLC increases with increasing NTAT. Figure 3 shows the effect of NTAT on TLC during the R8 stage of the indicated cultivars. The regression slope of Bengal was less steep than all other cultivars, suggesting lower susceptibility of Bengal to NTAT impacts on TLC. Results of the current study support the results of Cooper et al (2008), who reported increased TLC in rice cultivars grown at a NTAT of 30°C, compared with 18, 22, and 26°C.

NTAT Effects on Functional Properties

PV and SV. Positive correlation coefficients (Table III) across all cultivars indicated that PV increased with increasing NTATs. Medium-grain cultivars generally exhibited weaker correlations of PV versus NT_{95} than did long-grain cultivars, such that PV in Bengal was significantly correlated ($r = 0.57$) to NT_{95} only during the R7 stage. Among long-grain cultivars, correlations between PV and NT_{95} varied in terms of which R stage resulted in the strongest correlation. Cypress exhibited its strongest correlation in R6; LaGrue was most strongly correlated in R7, whereas Wells and XL723 were most strongly correlated in R8. Again, variations in cultivar kernel development patterns may be responsible for this variation in R stage correlation.

Figure 4 illustrates the effect of NTATs occurring during the R8 stage on PV. Regression slopes representing PV versus NT_{95} for all cultivars were similar within this particular R stage, on average indicating a change in viscosity of 9.3 RVU for each unit increase in NT_{95}. Individually, Cypress was most susceptible to changes in PV, exhibiting a slope of 10.4, whereas XL723 was least susceptible, with a slope of 7.6.

A large SV, or large difference between FV and PV, is generally considered a superior processing characteristic (Bergman et al 2004). In the current study, FVs varied considerably among cultivars and, in general, were not significantly correlated with NT_{95} (data not shown). However, SVs decreased significantly as NT_{95} increased. Correlations between SV and NT_{95} were strong-

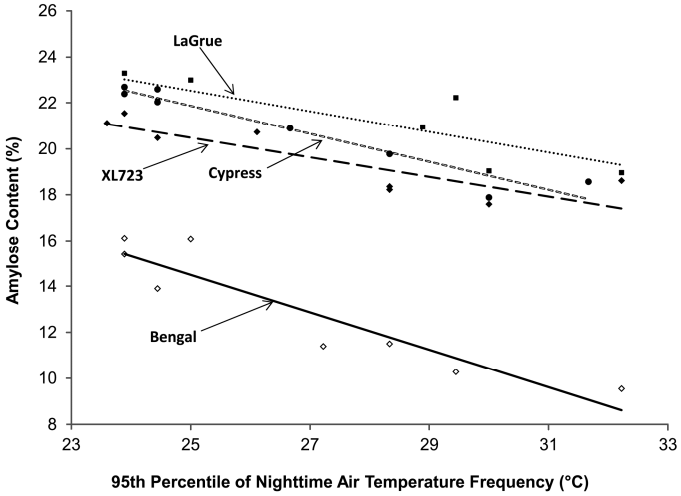

Fig. 1. Relationships of head rice amylose contents and 95th percentiles of nighttime air temperature frequencies during the R8 stages of the indicated cultivars grown during 2009 and 2010.

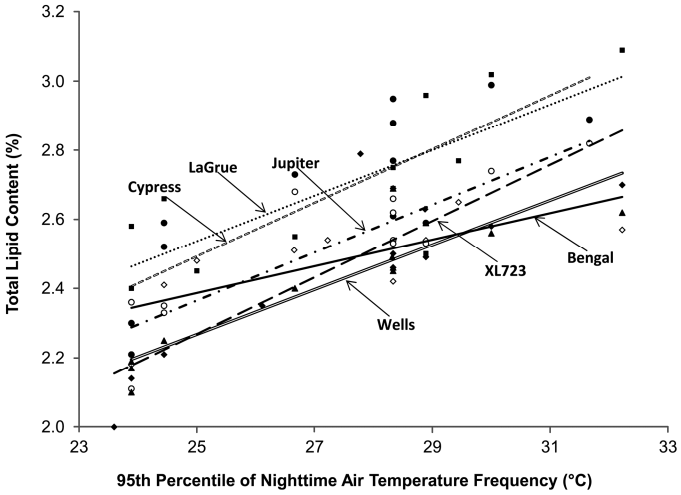

Fig. 3. Relationships of brown rice total lipid contents and 95th percentiles of nighttime air temperature frequencies during the R8 stages of the indicated cultivars grown from 2007 to 2010.

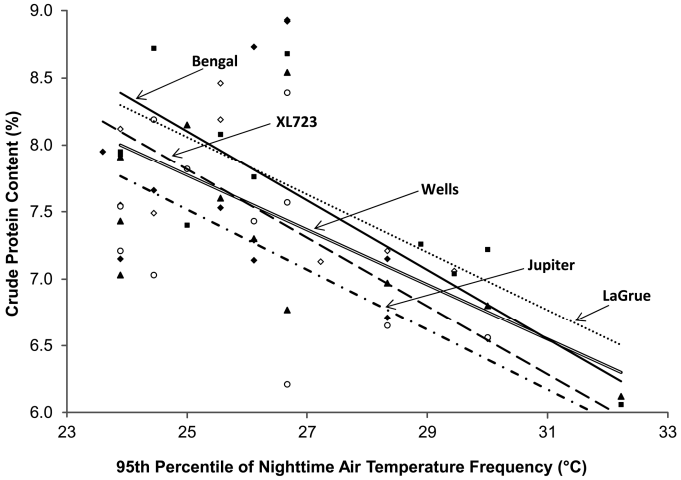

Fig. 2. Relationships of brown rice crude protein contents and 95th percentiles of nighttime air temperature frequencies during the R8 stages of the indicated cultivars grown from 2007 to 2010.

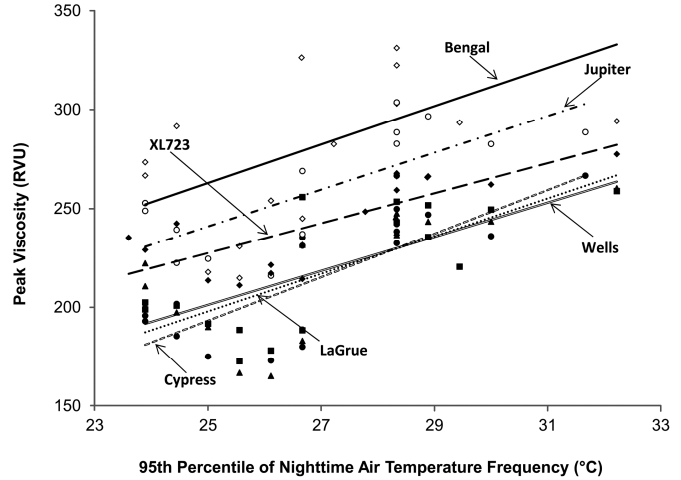

Fig. 4. Relationships of head rice peak viscosities and 95th percentiles of nighttime air temperature frequencies during the R8 stages of the indicated cultivars grown from 2007 to 2010.

est during the R7 stage for all cultivars except Cypress and Wells, which exhibited slightly stronger correlations in R6 (Table III).

Figure 5 illustrates the relationship between NT_{95} during the R8 stage and resulting SVs. Quadratic relationships were observed in the long-grain cultivars, whereas medium-grain cultivars displayed linear relationships between SV and NT_{95}. Medium-grain cultivars showed rapid and consistent SV reduction as NTAT increased, whereas long-grain cultivars were most susceptible to NT_{95} values above 27°C. Similar results were reported from a three-year field study by Dang and Copeland (2004), who observed greater SV for cultivars grown in cooler seasons than in relatively warmer ones. SV, an indicator of the degree of granule swelling after starch retrogradation, is mainly affected by linear-structured amylose, which retrogrades more easily than does branch-chained amylopectin (Dang and Copeland 2004). Therefore, it is likely that the observed reductions in SV resulted from the decreases in AC associated with increasing NTATs during kernel development.

GT. Positive correlations of GT_{onset} with NT_{95} during the R6, R7, and R8 stages (Table III) indicate increasing GTs with increasing NTATs during the critical grain-filling stages. Again, strong correlations were observed among the cultivars tested, but the R stage in which the strongest correlations occurred varied.

Figure 6 shows the increase in GTs with increasing NTATs for the indicated cultivars during their respective R8 stages. As expected, medium-grain Bengal exhibited a lower GT than did the long-grain cultivars tested, because of its lesser AC; however, the rate of increase in GT_{onset} per unit increase in NT_{95} was not significantly different among cultivars. The average rate of increase across all cultivars was 0.5, indicating that GT increased by 0.5°C with every unit increase in NT_{95}. Similar observations were made by Suzuki et al (2003), who reported that lower environmental temperatures significantly decreased onset, peak, and conclusion GTs, as well as gelatinization enthalpies, of four rice cultivars with varying ACs. Because increased GT results in greater temperature requirements to gelatinize starch, exposure to elevated NTATs has significant implications on end-use processing operations. Fitzgerald et al (2009) reported that increased GT can increase cooking duration and result in unacceptable cooked rice texture.

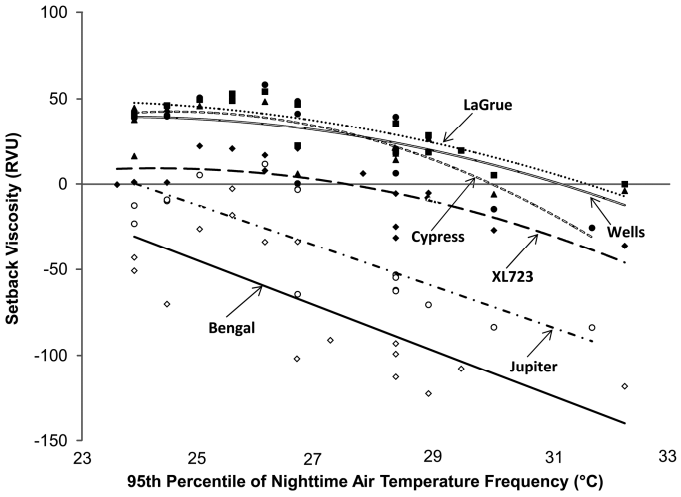

Fig. 5. Relationships of head rice setback viscosities and 95th percentiles of nighttime air temperature frequencies during the R8 stages of the indicated cultivars grown from 2007 to 2010.

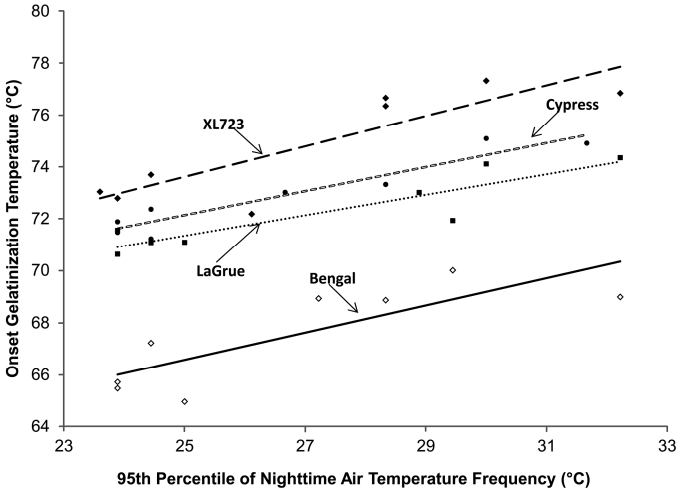

Fig. 6. Relationships of head rice onset gelatinization temperatures and 95th percentiles of nighttime air temperature frequencies during the R8 stages of the indicated cultivars grown during 2009 and 2010.

CONCLUSIONS

The present study has implications for rice physiologists, breeders, and processors. Rice physicochemical and functional properties were strongly correlated with NTATs that occurred during different reproductive stages. Elevated NTATs during critical grain-filling stages generally resulted in decreased AC and CPC, along with increased TLC, across the medium- and long-grain cultivars evaluated. These proximate changes in turn affected functional properties. PV increased, SV decreased, and GT_{onset} increased with increasing NT_{95} values.

By analyzing individual cultivars in isolation, the outcomes of this study were well defined. However, in commercial applications, in which rice lots are blended from a variety of cultivars, growing locations, and harvest dates, the degree of variation resulting from NTAT levels may be extremely difficult to predict or quantify. This variation may result in poor or inconsistent finished-product quality. The findings of this four-year study offer a possible explanation for the variation in quality that often affects end-use processing. Understanding the integral role that NTAT plays in rice quality variation is critical to minimizing its effects. This goal may best be achieved through a multisystems approach that includes breeding programs targeting the development of cultivars that are more stable over a range of growing conditions, production best practices that minimize the risk of exposure to elevated temperatures during the grain-filling stages, and procurement and processing options for processors.

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