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## Impact of field-scale nighttime air temperatures during kernel development on rice milling quality

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### ABSTRACT

Recent research has shown that elevated nighttime air temperatures (NTATs) may contribute to increased chalk and reduced milling quality. The objective of this study was to develop a method to quantify the effects of elevated NTATs on chalk formation and peak head rice yield (pHRY) in field-grown rice cultivars. To do so, 95th percentiles of NTAT frequencies (NT<sub>95</sub>) occurring during reproductive (R) stages of Bengal, Jupiter, Cypress, LaGrue, Wells, and XL723 cultivars were correlated with chalk levels and pHRYs observed during the 2007 through 2009 harvest seasons. Chalk levels were strongly correlated with NT<sub>95</sub> during the R7 and R8 stages for all cultivars, except Bengal. Peak HRYs of Cypress, LaGrue, Wells, and XL723 were linearly and inversely related to NT<sub>95</sub> occurring during the R8 stage, while pHRYs of Bengal and Jupiter showed no significant correlations with this percentile at any R-stage. Although strong correlations of chalk levels and pHRYs with NT<sub>95</sub> were observed during the R8 stage of cultivar development, it is speculated that rice plants classified in this stage actually exhibit many kernels that lag in development and exist in the R6 and R7 grain-filling stages, where elevated NTATs are thought to have deleterious effects on chalk levels and milling quality.

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### 1. Introduction

Globally, rice (*Oryza sativa*, L.) is the single most important food crop in terms of human food consumption, providing over 21% of the world's caloric needs (Fitzgerald et al., 2009). As such, rice industry sustainability is tied directly to field yields and quality, as rice is predominantly consumed as a direct, human food source. However, a growing body of research indicates that increasing global temperatures have a deleterious effect on rice yield and quality. Elevated growing temperatures have been correlated to decreased photosynthetic rate, spikelet fertility, seed yield (Prasad et al., 2006), biomass and growth (Ziska et al., 1996), and the ability to compete with weeds under high-temperature stress (Alberto et al., 1996).

Peng et al. (2004) emphasized that nighttime air temperature (NTAT) had a more pronounced negative effect on field yield of rice and other crops than daytime temperature. The study reported a reduction in rice yield of 10% for every 1 °C increase in tempera-

ture and an overall increase of 1.13 °C in nighttime temperatures, which were recorded by the International Rice Research Institute (IRRI) between 1992 and 2003. Later studies, carried out on rice cultivars under controlled conditions, have shown that elevated night temperatures ranging from 21 °C to 32 °C resulted in decreased yields (Nagarajan et al., 2010), decreased pollen germination, increased spikelet sterility, and increased respiration rates (Mohammed and Tarpley, 2009, 2010). Cheng et al. (2010) investigated the effect of carbon and nitrogen assimilation in rice plants in a controlled-environment study, concluding that night respiration was significantly increased by elevated NTATs, which could adversely affect grain yields. Morita et al. (2005) compared NTATs of 22 °C and 34 °C in a controlled-environment greenhouse study. Results indicated that the greater NTAT led to reductions in grain mass, kernel growth rates, and endosperm cell size.

Warm temperatures (generally >22 °C) are reported to interfere with metabolic processes during developmental stages in rice, affecting yield, as well as pre- and post-harvest quality. Chalkiness, a major quality defect in rice kernels, has been reported to occur due to environmental and genetic reasons (Lisle et al., 2000), and more specifically, is postulated to be induced by elevated temperatures during critical developmental stages of rice growth (Cooper

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et al., 2008; Fitzgerald and Resurreccion, 2009). Chalk is formed when irregular packing of starch granules (amyloplasts) occurs during the grain-filling stages of reproductive development, creating air spaces in the endosperm of rice kernels that result in opaque white regions of the kernel (Ashida et al., 2009). Cheng et al. (2005) suggested that the grain-filling stage is subject to the activity of various enzymes, primarily, temperature-sensitive grain-bound starch synthase (GBSS). The study showed that GBSS activity during grain-filling stages was greater at 22 °C than at 32 °C. Cooper et al. (2008) further proposed that increased chalk formation and decreased amylose content in rice could be due to a reduction in the rates of enzymatic activity and physiological functioning during high-NTAT exposure.

Lisle et al. (2000) observed differences in functional properties, such as pasting viscosities, of rice grown under different NTATs. The study concluded that both superior and inferior (chalky) kernels may develop on a single panicle of a plant, as a result of exposure to differing NTATs during their growth cycles. Fitzgerald and Resurreccion (2009) conducted a controlled-environment study to evaluate chalk formation by exposing rice samples to 19 °C and 26 °C daily minimum temperatures during their grain-filling stages. Results indicated that the warmer temperature did induce chalkiness. The study postulated that a decreased supply of substrates not only affects the grain-filling process, but also decreases the expression of genes that control enzyme activity aiding starch synthesis.

Chalkiness in rice kernels degrades the overall appearance of milled rice and generally results in lower HRY because chalky kernels tend to be weaker and more prone to breakage during milling than translucent kernels (Lisle et al., 2000; Kadan et al., 2008). Head rice yield is a widely accepted indicator of milling quality, defined as the mass percentage of rough rice kernels that are at least three-fourths of their original kernel length after milling (USDA, 1997). Greater HRY corresponds with greater economic value of rice (Siebenmorgen et al., 2008). Recent research relating chalk to NTATs also indicates strong correlation between HRY and NTAT. In a study by Cooper et al. (2006), a 17-year weather data set was used to correlate average daily low and daily high temperatures occurring during individual, projected reproductive growth stages to HRY of two long-grain cultivars. The study indicated that elevated NTATs during the R8 reproductive stage (Counce et al., 2000) reduced HRY in both cultivars. In a follow-up study, Cooper et al. (2008) reported that rice cultivars grown in controlled-environment growth chambers showed different degrees of susceptibility to high NTATs with respect to chalkiness and HRY. Among the cultivars used in the study, HRYs of long-grain cultivar, Cypress, and medium-grain cultivar, Bengal, were least affected by NTAT levels, while long-grain cultivar, LaGrue, was highly susceptible.

Most of the studies cited above used growth chambers to simulate environmental conditions, and did not necessarily represent field conditions, in which irregular and non-systematic temperature fluctuations occur. Therefore, this study was undertaken to assess the effects of NTATs during kernel development on chalk and HRY of field-grown rice samples. A method to quantify and correlate the occurrence of elevated NTATs to HRY and chalk was developed and used to indicate the reproductive growth stages in which susceptibility to elevated NTATs was apparent in several current cultivars.

## 2. Materials and methods

### 2.1. Sample production and procurement

Experimental data were obtained from the Arkansas Rice Performance Trials during harvest years 2007 through 2009. These trials were conducted at multiple locations throughout Arkansas,

U.S. Management practices, including planting dates, flooding, fertilization, and pesticide applications, were carried out to achieve near optimum yields across a wide range of Arkansas conditions. Nitrogen rates varied, depending on conditions, from 120 to 165 kg N ha<sup>-1</sup>; in general, these levels were sufficient to produce near optimum rice grain yield and quality without producing excessive growth.

Three long-grain (Cypress, LaGrue, and Wells) and two medium-grain (Bengal and Jupiter) pureline cultivars and a long-grain hybrid (XL723) cultivar, were grown each year from 2007 to 2009. Growing locations varied slightly throughout the three-year period, as follows: Corning, Newport, Stuttgart, and Rohwer, Arkansas in 2007; Corning, Pine Tree, Stuttgart, and Rohwer, Arkansas in 2008; and Keiser, Pine Tree, Stuttgart, and Rohwer, Arkansas in 2009. Growing locations were selected to span from northern latitude (36.4 N) to southern latitude (33.8 N) in Arkansas, thus representing increasing probability of NTAT severity during reproductive stages of rice plant growth. Using a randomized block design, a total of eighteen 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 the rate of 428 seeds/m<sup>2</sup> in a nine-row (0.18-m spacing) wide plot, 4.57-m in length. The hybrid cultivar was sown in plots of the same dimensions at the rate of 171 seeds/m<sup>2</sup>.

In each of the study years, samples of each cultivar were harvested over a range of moisture content (MCs) for each year/location/cultivar combination. The number of lots harvested during a given year depended on prevailing field drying conditions during the harvest season (Table 1). Harvest MCs ranged from 11.4<sup>1</sup> to 28.6% in 2007; 12.7 to 26.9% in 2008; and 13 to 28.9% in 2009.

Throughout the study, each harvested sample comprised 120 randomly selected, hand-cut panicles. Five of these panicles were stripped of their kernels for MC determination in the field, using a single kernel moisture meter (CTR 800E, Shizuoka Seiki Co., Ltd., Fukurui City, Shizuoka, Japan), calibrated with MCs measured by forced-air oven at 130 °C for 24 h. Each MC measurement comprised 300 rough rice kernels. Kernels from the remaining panicles, sufficient to yield at least 600 g of rough rice, were mechanically threshed in a portable thresher (SBT, Almaco, Nevada, IA), placed in canvas sample bags, and transported to the University of Arkansas Rice Processing Program pilot plant, Fayetteville, AR. Harvested rice samples were cleaned with a dockage tester (Carter-Day Dockage Tester, Carter-Day Co., Minneapolis, MN) and dried in a temperature- and humidity-controlled chamber (AA5582, Parameter Generation & Control, Inc., Black Mountain, NC) maintained at 25 °C and 53% relative humidity, corresponding to a rough rice equilibrium MC of approximately 12.5% (ASAE, 2007). Actual, dried rough rice MCs ranged from 11.8% to 12.4%, determined using a convection oven (1370FM, Sheldon Manufacturing, Inc., Cornelius, OR) in which triplicate, 15-g samples were dried for 24 h at 130 °C. After drying, samples were stored in Ziplock<sup>TM</sup> plastic bags at 4 °C until milling.

### 2.2. Reproductive growth staging

During each growing season, physiological stages of rice development for all cultivars were either visually identified, or estimated from weather data, according to a staging system defined by Counce et al. (2000). This rice staging system comprises vegetative stages (V), delineated by leaf development and reproductive stages (R), classified by the development of the main stem panicle. In this study, the staging process started with stage R2, which refers to

<sup>1</sup> All moisture contents are expressed on a wet basis unless otherwise specified.

**Table 1**  
Summary of lots analyzed for chalk<sup>a</sup> and peak head rice yield (pHRY)<sup>b</sup> for each year, location, and cultivar harvested from 2007 to 2009.

Year	Locations <sup>c</sup> (°latitude)	Cultivars										Total Chalk lots	Total pHRY lots		
		Bengal		Jupiter		Cypress		LaGrue		Wells				XL723	
		Chalk	pHRY	Chalk	pHRY	Chalk	pHRY	Chalk	pHRY	Chalk	pHRY			Chalk	pHRY
2007	Corning (36.4°N)	9	3	11	3	10	6	11	4	14	1	12	3	206	80
	Newport (35.6°N)	9	3	6	3	9	3	9	3	10	3	15	3		
	Stuttgart (34.5°N)	5	3	5	2	12	4	4	2	10	6	6	3		
	Rohwer (33.8°N)	8	2	4	3	3	5	6	3	11	5	7	4		
2008	Corning (36.4°N)	9	1	17	2	8	3	3	2	18	4	11	3	215	79
	Pine Tree (35.1°N)	5	3	6	4	2	3	4	4	6	3	2	3		
	Stuttgart (34.5°N)	18	3	13	4	15	3	10	2	13	3	13	3		
	Rohwer (33.8°N)	7	3	5	3	8	6	6	5	5	4	11	5		
2009	Keiser (35.7°N)	2	3	2	3	2	3	3	3	6	3	3	3	209	90
	Pine Tree (35.1°N)	9	3	9	3	9	3	9	3	9	3	6	3		
	Stuttgart (34.5°N)	15	3	12	3	15	3	12	6	12	3	14	3		
	Rohwer (33.8°N)	9	3	9	3	12	6	9	6	9	6	12	9		

<sup>a</sup> Lots used for chalk analysis were harvested within the following moisture content ranges: 11.4–28.6% (2007); 12.7–26.9% (2008); and 13.0–28.9% (2009).

<sup>b</sup> Lots used for pHRY were harvested within the optimal moisture content ranges of 19–21% for long-grain and 22–24% for medium-grain cultivars, according to Siebenmorgen et al. (2007).

<sup>c</sup> All locations are in AR, USA.

emergence of the flag leaf collar. The panicle exertion stage, or R3, begins with panicle exertion through the collar of the flag leaf on the main stem. R3 is also known by practitioners as the “heading date”. The “flowering” stage, or R4, indicates that one or more florets on the main stem panicle have reached anthesis. The R5 stage spans the time period when at least one caryopsis on the main stem elongates from initial kernel formation to final elongation, reaching the tip of the hull. The start of the grain-filling stage is termed R6 and is determined when at least one caryopsis on the main stem panicle has completely lengthened to the end of the hull. The stages in which one yellow hull and one brown hull appear on the main stem panicle are termed R7 and R8, respectively. The R9 stage is reached when all individual kernels that had reached R6 have reached R8.

During this study, the reproductive stages from R2 to R9 of each of the six selected cultivars were visually identified through daily monitoring of each cultivar’s reproductive development at the Stuttgart, AR location. During the R2 stage, panicles judged to be at the beginning of R2 were tagged with plastic markers and assigned an identifying number. Subsequently, these culms were monitored daily for the initiation of R3 (panicle emergence). Those panicles were monitored daily for each successive stage until R9 was reached. The day-of-year (DOY) for each identified R-stage of each cultivar was therefore recorded, as were ambient temperatures throughout all reproductive stages. From these temperatures, thermal units were calculated (see below) and thermal unit vs. reproductive staging sequences were tallied for each cultivar.

At locations other than Stuttgart, the “50% heading date”, the date upon which 50% of the panicles in a field were judged to have emerged, was visually determined for each field plot and was deemed the date of R3 stage initiation. The DOY of its occurrence was recorded for each of the six cultivars. For these locations, subsequent stages and DOYs of their initiation were estimated using ambient temperatures collected at each location and thermal unit vs. reproductive staging data, as described below.

### 2.3. Weather data and thermal unit calculation

Thermal units are often used to model the progression from one crop development stage to another. A thermal unit represents the product of temperature and the duration over which the temperature occurs. Rice development has successfully been modeled using thermal units by Downey and Wells (1975) and has been used extensively in southern U.S. rice production areas

to predict rice plant development. The computations used by Downey and Wells to predict several stages of rice development employ daily maximum and minimum temperatures. The rice growth staging system of Counce et al. (2000) was developed to provide objective identification of plant developmental features that can be recognized by different people observing the same plants. This rice growth staging system also allows shorter durations to be used in calculating thermal units (Clements et al., 2003; Watson et al., 2005). Purcell (2003) showed the value of using hourly rather than daily time steps for thermal unit calculations. Therefore, for this study, ambient temperature was recorded in 30-min increments using two temperature sensors (HOBO Pro/Temp Data Logger, Onset Computer Co., Bourne, MA) positioned at each growing location. Based on these 30-min temperatures, and using the following equation, degree-day-10 (DD10) thermal units (°C-day) over the course of each day were quantified.

$$DD10 = \sum_{i=1}^{48} \left[ \left\langle \frac{(T_{MAX} (^{\circ}C) + T_{MIN} (^{\circ}C))}{2} \right\rangle - 10^{\circ}C \right]_{30\text{-min}} \times 0.5 \text{ h} \times \frac{1 \text{ day}}{24 \text{ h}} \quad (1)$$

- $T_{MAX}$  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.

Thermal unit accumulation at the initiation of the R3 stage was assigned a value of zero. DD10 values were computed using the 30-min temperature data and accumulated to determine the progression through R-stages. To determine the thermal units required for a rice cultivar to advance from one R-stage to another, the accumulated DD10 values computed from the temperature readings at Stuttgart, AR, were first aligned with the DOYs observed for each stage initiation at Stuttgart; this process was repeated for each cultivar in each study year. The rate of R-stage development for each cultivar was assumed to be constant across locations during the same growing season. Thus, the thermal unit accumulation vs. R-stage progression rate that was determined for each cultivar in each year at Stuttgart was utilized to determine the DOY initiations of each R-stage at the other locations, using the visually observed R3 DOYs and the 30-min temperature data for each cultivar at each location as starting data. This process yielded the DOY initiation and duration of each cultivar’s R-stages at each location in each year, from which subsequent nighttime temperature analysis was based.

#### 2.4. Chalk

Rough rice (100 g) from each harvest lot (Table 1) was de-hulled to produce brown rice. Chalk measurements were performed in duplicate on 100-kernel brown rice sets per harvest lot for each harvest year/location/cultivar combination. Brown rice kernels were placed on a tray (152 mm × 100 mm × 20 mm) made from 32 mm-thick, clear acrylic sheet, so that no single kernel touched another kernel. A digital image of kernels was created by placing the tray on the scanner of an image analysis system (WinSeedle Pro 2005a™, Regent Instruments Inc., Sainte-Foy, Quebec, Canada). Prior to measurements, the imaging system was configured to color-classify chalk by selecting and scanning a brown rice kernel considered to be completely chalky into the imaging system as a reference color for chalk. The imaging system measured and recorded the number of pixels representing the entire kernel area from the scanned images, as well as number of pixels corresponding to those areas color-classified for chalk on a kernel. Percent chalk in a sample was determined as the ratio of the total chalky area (pixels) of the 100-kernel set to the total area of the kernels, multiplied by 100.

In 2007, chalk values were observed to be greater in rice harvested at low HMCs, when fewer immature kernels were present and chalk presence was readily apparent. In 2008 and 2009, when chalk formation was lower than in 2007, few differences in chalk values were observed among cultivars harvested across all HMCs. It is speculated that when harvesting at high average MCs, affected kernels may not yet have matured to the point where chalk manifests. At lower average harvest MCs, the endosperm of such kernels has likely developed to yield visible opaque areas. Hence, in this study, chalk analyses were carried out in duplicate on all harvest lots (Table 1), and chalk values were averaged across all sample lots within each harvest year/location/cultivar combination.

#### 2.5. Head rice yield

Head rice yield analysis for the current study included only those lots in which samples were harvested within optimal MC ranges, as described by Siebenmorgen et al. (2007) (Table 1). Optimal HMC ranges in Arkansas were found to be 19–21% for long-grain and 22–24% for medium-grain cultivars. Head rice yields obtained from these lots were considered to be peak HRYs (pHRYs) for each year/location/cultivar combination. The use of pHRYs in the current study was designed to minimize the effects of immature kernels associated with high MCs and fissuring associated with low MCs, thereby focusing on the effects that NTAT had on milling quality.

Prior to milling, samples were removed from storage and equilibrated at room temperature for at least 24 h. For each milling test, duplicate 150-g rough rice samples were de-hulled in a laboratory sheller (THU, Satake, Tokyo, Japan) with a clearance of 0.048 cm (0.019 in.) between the rollers. The resultant brown rice samples were milled for 30 s using a laboratory mill (McGill No. 2, RAPSCO, Brookshire, TX). Head rice was then separated from broken pieces using a double-tray sizing device (Seedburo Equipment Co., Chicago, IL). Head rice yield was expressed as the mass percentage of the 150 g of rough rice that remained as head rice. Peak HRY was calculated as the mean of these duplicate sample HRYs. To account for differences in degree of milling (DOM), pHRYs were adjusted to a 0.4% surface lipid content per the methods described below.

#### 2.6. Surface lipid content

Surface lipid contents of head rice obtained from 2007 and 2009 harvest lots were determined in duplicate using a lipid extraction system (Soxtec Avanti 2055, Foss North America, Eden Prairie, MN) according to the method of Matsler and Siebenmorgen (2005). In brief, 5 g of head rice were weighed into cellulose thimbles (Foss

North America, Eden Prairie, MN); the thimbles and kernels were pre-dried for 1 h in a 100 °C oven. Subsequently, lipid was extracted from the sample using 70 ml of petroleum ether (boiling point 35–60 °C; VWR, Suwanee, GA). Samples were boiled in solvent for 20 min over a 135 °C hot plate, rinsed with petroleum ether condensate for 30 min, and dried for 5 min. After the extraction cycle, the extraction cups were removed from the Soxtec unit and placed into an oven maintained at 100 °C for 30 min to evaporate the solvent. The extraction cups were placed in a desiccator at room temperature for approximately 30 min to cool before weighing. The difference between the mass of the cups containing the extracted lipid and the original empty cup mass was calculated to obtain the mass of the extracted lipid. Surface lipid content was expressed as the mass percentage of extracted lipid mass to the original rice mass.

Surface lipid contents of samples from harvest year 2008 were measured using a diode array NIR analyzer (DA 7200, Perten instruments, SE-141 05 Huddinge, Sweden), which was calibrated to Soxtec lipid extraction system SLCs measured in previous years. Three scans were conducted for each sample and average SLC values were calculated.

All pHRY values used in this study were adjusted to account for differences in DOM according to the method developed by Pereira et al. (2008), which maintains that HRY changes by 1.13 percentage points (pp) for every 0.10 pp change in SLC in long-grain cultivars and 0.85 pp in medium-grain cultivars. For the current study, an arbitrary standard SLC of 0.4% was used.

#### 2.7. Nighttime air temperature analysis

Ambient temperatures were recorded throughout each growing season in all locations. Those recorded during the time of the day extending from 8:00 pm to 6:00 am were considered as NTATs. Frequencies of the NTATs during each R-stage were first tallied and the 95th percentiles of NTAT frequency (NT<sub>95</sub>) during each R-stage, below which 95% of the NTATs occurred, were calculated for all cultivar/location/year combinations using a cumulative frequency distribution model (JMP release 8.2, SAS institute, Cary, NC). Chalk and pHRY values were plotted against the NT<sub>95</sub> during each R-stage. The statistical significance of the correlations was determined by analysis of variance at  $\alpha = 0.05$  using polynomial regression analysis (JMP release 8.2, SAS institute, Cary, NC). Pair-wise correlation coefficients between pHRY and chalk and corresponding NT<sub>95</sub> during an R-stage for each year, location, and cultivar were determined using a multivariate analysis (JMP release 8.2, SAS institute, Cary, NC).

### 3. Results and discussion

Average chalk values and pHRYs of cultivars harvested during 2007–2009 are shown in Table 2, for informational purposes only. General observations show that harvest year 2007 produced greater chalk levels and lower pHRYs than 2008 and 2009 in all cultivars except Bengal. Southern locations Rohwer (33.8N) and Stuttgart (34.5N) usually exhibited greater chalk levels and lower pHRYs in most cultivars than those grown in northern locations (35.1N–36.4N) during all harvest years.

Fig. 1a and b illustrates NTAT frequencies, which were used to calculate NT<sub>95</sub>, during the R7 and R8 stages of XL723 grown at Stuttgart, AR in 2007–2009. Also displayed are the corresponding mean chalk and pHRYs that were recorded during each year (inset table). In the R7 stage (Fig. 1a), greater frequencies of NTATs above 25 °C were observed in 2007 than in 2008 or 2009. These greater temperature frequencies corresponded to greater chalk and lower pHRYs. Conversely, greater frequencies of temperatures

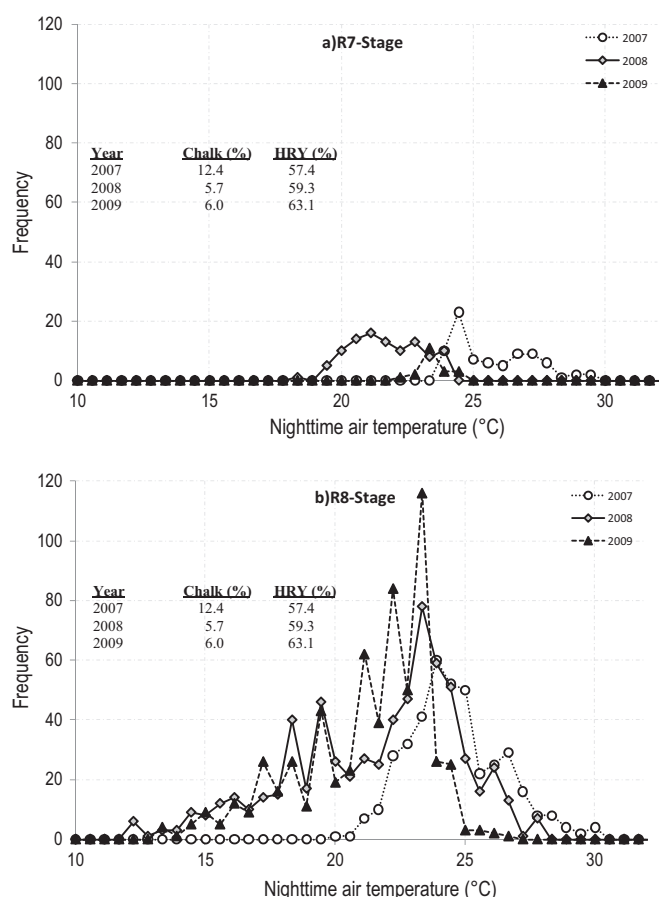
**Table 2**  
Chalk values<sup>a</sup> and peak head rice yields (pHRYS)<sup>b</sup> for cultivars harvested from different locations from 2007 to 2009.

Year	Location <sup>c</sup>	Cultivars											
		Bengal		Jupiter		Cypress		LaGrue		Wells		XL723	
		Chalk (%)	pHRY (%)	Chalk (%)	pHRY (%)	Chalk (%)	pHRY (%)	Chalk (%)	pHRY (%)	Chalk (%)	pHRY (%)	Chalk (%)	pHRY (%)
2007	Corning	3.88	63.5	5.36	63.2	4.61	62.9	10.1	47.2	8.27	54.5	11.8	52.2
	Newport	3.03	69.1	2.44	66.4	3.22	62.4	4.77	57.8	4.39	58.5	7.20	60.9
	Stuttgart	5.12	66.0	3.40	65.6	6.41	60.5	11.1	53.7	8.82	58.7	12.4	57.4
	Rohwer	3.33	64.3	3.26	63.5	4.01	63.7	5.38	52.2	5.28	57.5	10.9	59.6
2008	SE	0.19	0.80	0.33	0.54	0.21	0.58	0.63	1.27	0.43	0.64	0.48	0.99
	Corning	3.27	65.6	1.27	65.5	1.94	64.3	2.95	60.6	2.55	62.0	4.89	61.8
	Pine Tree	2.91	65.5	1.35	65.4	2.06	68.1	2.04	62.1	2.04	63.1	4.49	63.0
	Stuttgart	3.22	64.7	1.74	65.0	3.80	59.1	3.27	58.4	3.06	58.7	5.67	59.3
2009	Rohwer	2.90	64.6	1.63	65.7	2.30	60.7	1.90	51.9	2.64	55.0	5.09	57.0
	SE	0.11	0.40	0.07	0.67	0.19	0.99	0.17	1.07	0.14	0.94	0.20	0.68
	Keiser	4.37	63.8	0.83	61.2	1.10	66.5	1.60	59.8	1.93	55.9	2.67	62.0
	Pine Tree	3.15	59.5	1.10	63.4	2.34	67.9	1.87	66.1	2.72	66.5	5.13	64.3
	Stuttgart	3.98	62.3	2.09	62.5	2.99	65.7	2.49	61.4	3.11	63.4	6.04	63.1
	Rohwer	3.35	65.8	1.40	64.8	2.75	65.9	2.77	62.8	2.91	63.1	5.67	63.7
	SE	0.15	0.64	0.10	0.52	0.14	0.34	0.11	0.52	0.10	0.84	0.25	0.26

<sup>a</sup> Chalk values were measured in duplicate and averaged across all harvest lots analyzed for each year/location/cultivar combination (Table 1).

<sup>b</sup> Peak HRYS were measured in duplicate and averaged across all lots harvested within the optimal moisture content ranges of 19–21% for long-grain and 22–24% for medium-grain cultivars, according to Siebenmorgen et al. (2007) (Table 1). Peak HRYS were adjusted to a 0.4% surface lipid content according to the method of Pereira et al. (2008).

<sup>c</sup> All locations are in AR, USA.



**Fig. 1.** Nighttime air temperature frequencies during the R7 (a) and R8 (b) reproductive stages of long-grain hybrid XL723 grown at Stuttgart, Arkansas in 2007, 2008, and 2009. Mean peak head rice yields (pHRYS) and chalk levels for each year are indicated (inset).

below 25 °C in 2008 and 2009 reflected lower chalk values and greater pHRYS. Although differences between years were not as great, similar trends were observed in the R8 stage, wherein NTAT frequencies were much greater, due to the extended duration of this stage (Fig. 1b). Other location/cultivar combinations exhibited similar results (data not shown), anecdotally suggesting that rice exposed to greater NTATs during the R7 and R8 stages corresponded to greater chalk levels and lower pHRYS. These observations agree with a previous study, in which Lisle et al. (2000) observed an increase in the number of chalky rice kernels grown at a daytime/nighttime temperature combination of 38/30 °C compared to rice grown at 26/20 °C.

Table 3 shows correlation coefficients at a 0.05 significance level that were calculated to describe significant relationships of chalk levels and pHRYS to NT<sub>95</sub> occurring during the R5 to R8 reproductive stages. Strong correlations were observed between chalk levels and NT<sub>95</sub> during the R6 stages of Cypress and LaGrue, while all cultivars except Bengal had strong correlations between chalk levels and NT<sub>95</sub> at the R7 and R8 reproductive stages. Cultivar Bengal showed no significant correlations between chalk and NT<sub>95</sub> at any R-stage. There were no correlations of chalk to NT<sub>95</sub> at the R5 stage for any cultivar.

Negative correlations between pHRYS and NT<sub>95</sub> during the R7 and R8 stages (Table 3) were observed in all long-grain cultivars, with the exception of Wells at the R7 stage, indicating a reduction in pHRYS with increasing NTAT. Strong correlations (−0.80 or greater) during either or both the R7 and R8 stages were observed in LaGrue, Wells, and XL723, suggesting an acute susceptibility of these cultivars to elevated NTAT effects. No correlations between pHRYS and NT<sub>95</sub> existed in medium-grain cultivars Bengal and Jupiter, suggesting the resistance of these cultivars to the effects of elevated NTATs on milling quality. Additionally, there were no correlations between pHRYS and NT<sub>95</sub> for any cultivar in the R5 and R6 stages.

Fig. 2a and b represents regression line trends in chalk levels vs. NT<sub>95</sub> during the R7 and R8 stages, respectively. The steeper slopes of LaGrue, Wells, and XL723, particularly in their R8 stages, indicate greater chalk formation susceptibility to NTATs compared to Cypress and Jupiter. No significant correlation of chalk level to NT<sub>95</sub> was observed in Bengal.

**Table 3**  
Correlation coefficients of chalk and peak head rice yield (pHRY) with the 95th percentiles of nighttime air temperature frequencies (NT<sub>95</sub>) during the R5 to R8 reproductive stages of long-grain and medium-grain rice cultivars grown in Arkansas from 2007 to 2009.

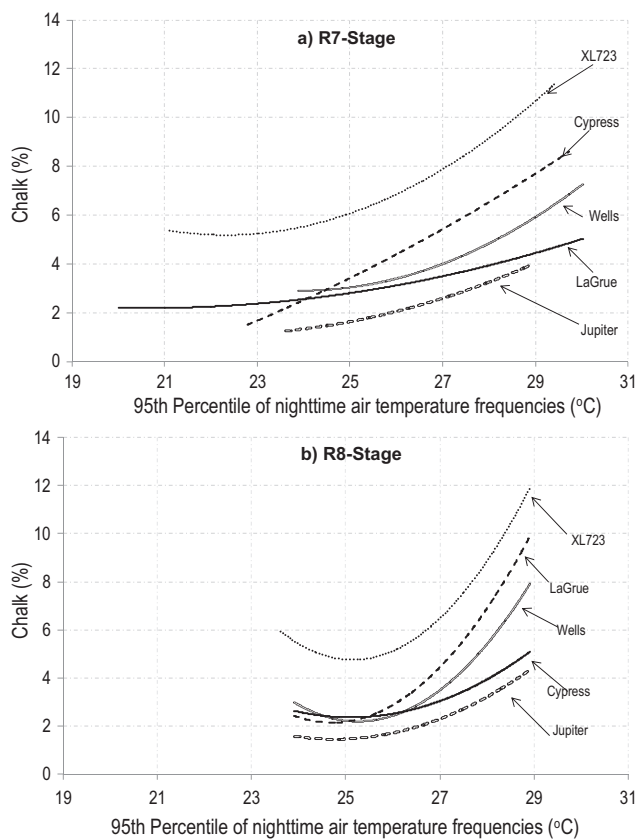
Year	Quality	R-stage	Cultivars						
			Bengal	Jupiter	Cypress	LaGrue	Wells	XL723	
2007–2009	Chalk	R5	ns <sup>a</sup>	ns	ns	ns	ns	ns	ns
		R6	ns	ns	0.79	0.60	ns	ns	ns
		R7	ns	0.73	0.68	0.72	0.68	0.67	0.67
		R8	ns	0.72	0.66	0.79	0.76	0.81	0.81
2007–2009	pHRY	R5	ns	ns	ns	ns	ns	ns	ns
		R6	ns	ns	ns	ns	ns	ns	ns
		R7	ns	ns	-0.62	-0.80	ns	-0.61	-0.61
		R8	ns	ns	-0.62	-0.80	-0.81	-0.80	-0.80

<sup>a</sup> Correlation coefficients were not significant.

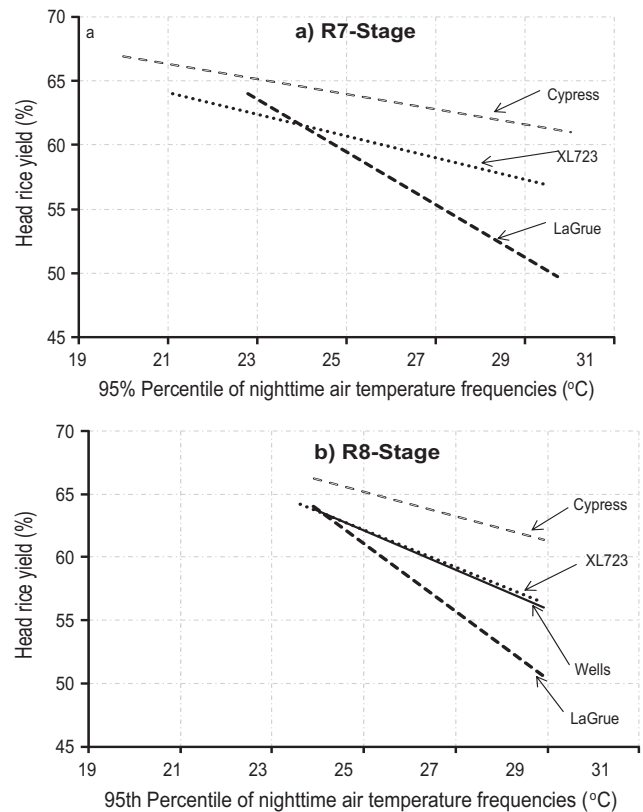
Fig. 2b reveals a quadratic relationship between chalk levels and NT<sub>95</sub> for XL723, LaGrue, and Wells cultivars, suggesting an optimum temperature below and above which chalk formation was triggered. In a controlled-temperature study, Yoshida and Hara (1977) observed a similar second-order relationship in chalk formation in indica (IR20) and japonica (Fujisaka 5) rice, wherein elevated chalk levels were observed at NTATs below and above 18 °C. This may suggest the possibility of temperature optima for enzyme activities responsible for packing of starch granules in the endosperm during the grain-filling stages of different rice cultivars. Counce et al. (2005) noted eight enzymes required to convert sucrose into fully branched starch molecules in developing rice kernels. Starch synthesis enzymes, particularly starch synthase, are known to be sensitive to temperatures above 25 °C, as observed in wheat and maize (Keeling et al., 1994).

The effects of NTATs during the R7 and R8 stages of cultivar development on pHRY are shown in Fig. 3a and b, respectively. LaGrue showed the most rapid decrease in pHRY with increased NT<sub>95</sub> during the R7 and R8 stages, followed by Wells and XL723; Cypress showed the least rapid decrease, which supports anecdotal observations and those of Siebenmorgen et al. (2007) that Cypress is a stable milling quality cultivar. Results similar to the findings of this study were observed by Cooper et al. (2008), wherein no significant changes in pHRY of Cypress were observed, while pHRY of LaGrue decreased significantly with increasing NTAT.

Kernel maturation, or progression through R-stages, is asynchronous across the kernels on a rice plant, and certainly across the kernels on the plants within a field (Holloway et al., 1995; Counce et al., 1996). This would indicate that a particular R-stage may not be representative of all kernels on a plant. For example, by definition, the R6 reproductive stage represents the stage in which the caryopsis of the first observed kernel on the main stem panicle completely



**Fig. 2.** Relationships of chalk values and 95th percentiles of nighttime air temperature frequencies during the R7 (a) and R8 (b) stages for the indicated long- and medium-grain cultivars grown during 2007, 2008, and 2009. No significant correlations were observed in Bengal.



**Fig. 3.** Relationships of peak head rice yields and the 95th percentiles of nighttime air temperature frequencies during the R7 (a) and R8 (b) stages of the indicated long-grain cultivars grown during 2007, 2008, and 2009.

elongates to the end of the hull (Counce et al., 2000). Subsequent kernels lag behind in the maturation process and pass through the grain-filling stage after this first kernel. Therefore, while the results of this study indicate that NTATs during the R7 and R8 stages are most prominent in affecting chalk and pHRY levels, the R6 grain-filling stage is hypothesized to be the developmental stage during which NTAT effects initiate chalk formation, resulting in reduced pHRYS. Generally insignificant correlations between both chalk and pHRY vs. NT<sub>95</sub> during the R5 and R6 stages (Table 3) suggest that, although the plant is classified in the R5 or R6 stage, the great number of less mature kernels that are present in the R3 and R4 stages are not significantly affected by NTATs. These speculations are supported by the findings of the historical analysis relating milling quality to NTATs by Cooper et al. (2006), in which it was similarly concluded that while NTATs were shown to affect kernels most prominently during the R8 stage, most of the kernels were actually reasoned to be in earlier reproductive (grain-filling) stages. Results of the current study also parallel the findings of Morita et al. (2005), which showed deleterious effects of high NTATs on grain quality during the middle to late stages of grain-filling.

#### 4. Conclusions

Chalk levels were directly correlated and pHRYS were inversely correlated to NT<sub>95</sub> during the R8, and to a lesser degree, R7, reproductive stages. However, neither chalk nor pHRY was significantly correlated to NT<sub>95</sub> during the R5 and R6 stages. Since the staging system developed by Counce et al. (2000) is based on a visual rating of the most mature kernel's development on the main stem panicle, strong correlations at the R8 stage may actually indicate that NTAT effects are incurred by kernels during the R6 and R7 grain-filling stages.

There were no significant correlations of chalk or pHRY with NT<sub>95</sub> for medium-grain Bengal. Medium-grain Jupiter showed no correlations between pHRY and NT<sub>95</sub>, but did show significant correlations between chalk and NT<sub>95</sub> during the R7 and R8 stages. This suggests that Bengal, and to some extent, Jupiter, were not susceptible to the impacts of the NTATs occurring during critical reproductive growth stages during this study. Among the long-grain cultivars, LaGrue, XL723, and Wells showed greater increases in chalk and greater reductions in pHRY with increasing NT<sub>95</sub> during the R7 and R8 stages than did Cypress. The results generally imply that the effects of elevated NTATs on chalk and milling quality are incurred during certain critical reproductive stages and are cultivar-specific.

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