

Effects of Preharvest Nighttime Air Temperatures on Whiteness of Head Rice

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ABSTRACT

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The effects of nighttime air temperature (NTAT) on the color of milled rice were investigated. Elevated NTATs occurring during the critical grain-filling stages of kernel development impacted the color of head rice. Six cultivars, grown at multiple field locations from northern to southern Arkansas during 2007–2010, were evaluated for head rice color, using whiteness (L^*) and yellowness (b^*) indices, and for chalk. Nighttime temperatures were recorded throughout production at each of the selected locations, and the 95th percentiles of NTAT frequencies (NT_{95}) were calculated for each cultivar's reproductive (R) stages. Head rice color values were analyzed in relation to NT_{95} occurring during

the grain-filling (R6–R8) stages and in relation to percent chalkiness. Whiteness generally increased with increasing NTAT and with increasing chalkiness. Yellowness decreased as chalkiness increased. Moreover, kernel whiteness increased even when measured in the absence of chalky kernels, suggesting that starch granule organization throughout the kernel, even in nonchalky portions, was altered, which could result in compromised physical integrity and processing functionality. Cultivars varied in their susceptibility to the effect of NTAT on color, as has been previously demonstrated with milling quality and functional properties.

Rice color is perceived by consumers as an important indicator of milled rice quality, and thus it impacts the commercial value of rice. Translucent white rice is generally preferred over rice with a gray or yellow cast in most markets. In the United States, the Federal Grain Inspection Service uses visual color as a qualification in both grade standards and degree of milling (DOM) classes (USDA 2005). Whiteness is used as an indicator of the degree to which rice is milled because DOM is a function of the residual lipids remaining on kernels after milling. These lipids impart brown color to the relatively white endosperm of rice kernels (Bergman et al 2004). Research shows that rice whiteness increases with increasing DOM, whereas head rice yield decreases (Chen and Siebenmorgen 1997; Siebenmorgen et al 2006; Saleh and Meullenet 2007). However, because whiteness values have been shown to plateau with increasing milling duration, overmilling may compromise yield with little to no gain in color quality (Lanning and Siebenmorgen 2011).

Certain rice cultivars are inherently darker or more prone to discoloration than others because of genetic differences (Belefant-Miller 2009). However, little information is available to explain the role that genetics plays in milled rice discoloration. Belefant-Miller (2009) induced yellowing in 98 southern U.S. rice cultivars in an effort to identify those that were resistant to yellowing, only to find that although there were distinctions between low- and high-yellowing varieties, the overall degree of yellowing was significant throughout the population.

It has also been anecdotally observed that rice lots of the same cultivar but from different crop years vary in color as a result of environmental growing conditions. A growing body of research indicates that preharvest climatic effects, specifically elevated nighttime air temperatures (NTATs) occurring during the grain-filling stages, have deleterious effects on kernel formation and resultant quality. Early studies showed strong correlations of yield decrease with NTATs above 24°C (70°F) during the grain-filling stages (Downey and Wells 1975), as well as reduced kernel dimensions and induced morphological damage in kernels, including loosely packed starch in the endosperm, manifested as chalk (Tashiro and Wardlaw 1991). 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 milling quality. Fitzgerald and Resurreccion (2009) also demonstrated that warm temperatures during the grain-filling stages induced chalkiness and suggested that elevated temperatures reduced the substrate supply from vegetative tissues, thereby inhibiting the expression of genes that control enzymes associated with starch synthesis. Most recently, a four-year field study of six cultivars grown throughout Arkansas illustrated that elevated NTATs during critical grain-filling stages affected physicochemical properties. Chalk and lipid contents were shown to increase with increasing NTAT, whereas amylose and protein contents were shown to decrease (Lanning et al 2011, 2012).

Although the aforementioned studies did not specifically evaluate color in relation to NTATs, it stands to reason that kernel color may be impacted by the same disruptions in kernel development that result in chalk and other defects, which could explain some of the cultivar and year variation cited by studies such as Belefant-Miller (2009). The following analysis, based on the same 2007–2010 data set used by Lanning et al (2011), was designed to evaluate the effects of NTAT on the color of milled rice.

MATERIALS AND METHODS

Sample Production

Three long-grain pure-line cultivars (Cypress, LaGrue, and Wells), two medium-grain pure-line cultivars (Bengal and Jupiter), and one long-grain hybrid cultivar (XL723) were grown as part of the Arkansas Rice Performance Trials system each year from 2007 to 2010. This program consisted of multiple growing locations throughout Arkansas, strategically selected to span from northern to southern latitudes to increase the probability of NTAT differences during the reproductive stages. Annual growing locations differed throughout the four-year period: 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, Rohwer, and Stuttgart in 2010. At each location, the six cultivars were planted in randomly assigned triplicate plots. Management practices included recommended planting dates, flooding, fertilization (nitrogen rates varied depending on conditions, from 120 to 165 kg/ha), and pesticide applications to achieve near-optimum yields (Slaton 2006).

Determination of 95th Percentiles of NTAT

The 95th percentiles of NTAT (NT_{95}) for each year, location, and cultivar R stage were calculated to represent the levels of NTATs observed. The method for calculating these values was described in detail by Ambardekar et al (2011). In brief, the procedures were as follows.

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In each of the four study years, ambient temperatures were recorded throughout the reproductive growing season. Two sensors (HOBO Pro/Temp Data Logger, Onset Computer, Bourne, MA, U.S.A.) were positioned at a height of ≈ 1.25 m above ground level and within 0.3 m of the rice canopy in the plots at each growing location. Each sensor recorded temperature data in 30 min increments. NTATs were considered to be those ambient temperatures recorded during the period from 8:00 p.m. to 6:00 a.m. each day.

A visual staging system (Counce et al 2000) was used to identify physiological stages of rice development for the cultivars grown in Stuttgart, Arkansas, each year. This rice staging system comprised vegetative stages, delineated by leaf development, and reproductive stages (R), classified by the development of the main stem panicle. Stages R6–R8 were termed the grain-filling stages and began when at least one caryopsis on the main stem panicle had completely lengthened to the end of the hull. In the current study, reproductive stages from R3 to R9 for each cultivar were identified through daily monitoring of each cultivar’s reproductive development (Ambardekar et al 2011). The day of year (DOY) upon which each R stage initiated was recorded. Ambient temperature data were used to calculate thermal units (Downey and Wells 1975), which were then accumulated for each cultivar R stage.

At other locations, the R3 stage for each cultivar was visually identified, and its initiating DOY was recorded. DOYs for subsequent stages were not visually identified but rather derived, based on the ambient temperatures collected at each location and the cultivar-specific thermal unit versus reproductive staging data that was collected from Stuttgart. The amount of thermal energy required to progress from one R stage to the next was assumed to be constant for each cultivar across locations during the same growing season.

Once the initiation DOYs and durations for stages R3–R9 were calculated for each cultivar, frequencies of NTATs during each R stage were used to determine NT_{95} values for all year/location/cultivar combinations, following a cumulative frequency distribution model (JMP release 8.2, SAS Institute, Cary, NC, U.S.A.). The NT_{95} value represented the temperature below which 95% of the observed NTATs occurred during a given year, location, and cultivar R stage.

Sample Procurement and Preparation

At each location during harvest years 2007–2009, samples of each cultivar were hand-harvested over a wide range of moisture contents (MCs) to evaluate the effects of harvest moisture content (HMC) on postharvest milling quality and functional attributes. The number of lots harvested during a harvest year depended on the HMCs targeted and the prevailing field conditions during the harvest season (Ambardekar et al 2011). Rice cultivars were harvested over an MC range of 11.4–28.6% (wb) in 2007, 12.7–26.9% in 2008, and 13.0–28.9% in 2009. In 2010, samples were harvested over a more narrow range of MCs, in which milling quality was maximized. Target ranges were 19–22% MC (wb) for

long-grain cultivars and 22–24% MC for medium-grain cultivars (Siebenmorgen et al 2007); actual MCs were 16.4–24.4 and 18.6–24.4% (wb), respectively, across all locations in 2010.

Each year, samples were harvested and cleaned according to the procedures described in Ambardekar et al (2011). The samples were dried in a temperature- and humidity-controlled chamber (AA5582, Parameter Generation & Control, Black Mountain, NC, U.S.A.) 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 with a convection oven (1370FM, Sheldon Manufacturing, Cornelius, OR, U.S.A.) in which triplicate 15 g samples were dried for 24 hr at 130°C. After drying, samples were stored in zippered plastic bags at 4°C until milling.

Duplicate 150 g rough rice subsamples from each location/cultivar/replication/HMC combination were dehulled 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 sec with a laboratory mill (McGill no. 2, RAPSCO, Brookshire, TX, U.S.A.) with a 1.5 kg weight on the lever arm situated 15 cm from the milling chamber (Andrews et al 1992; Bennett et al 1993). Head rice, composed of milled kernels at least three-quarters of their original length (USDA 2005), was then separated from broken pieces with a double-tray sizing device (Seedburo Equipment, Chicago, IL, U.S.A.).

Chalk

A rough rice sample of 100 g from each harvest lot was dehulled to produce brown rice. Chalk measurements were performed in duplicate on 100-kernel brown rice sets for each harvest year/location/cultivar/replication/HMC combination. Brown rice kernels were placed on a tray (152 × 100 × 20 mm) made

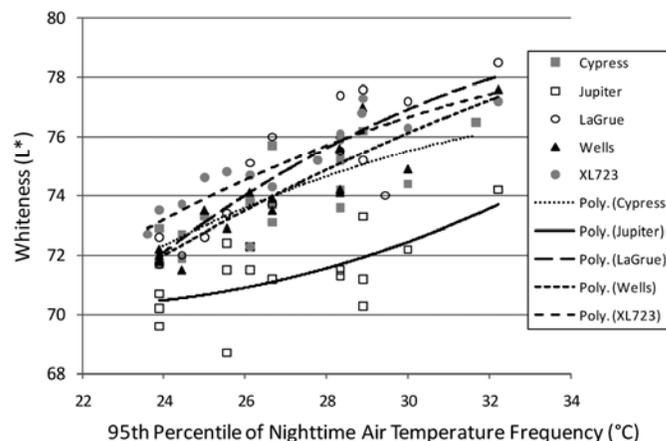


Fig. 1. Relationship of whiteness (L^*) values and 95th percentiles of nighttime air temperature frequencies during the R8 stage for the indicated cultivars grown from 2007 to 2010. Cultivars for which this relationship was not statistically significant (Table II) are not included.

TABLE I
Nighttime Air Temperatures (NTATs, °C) Recorded During the R6–R8 Stages, Averaged Across All Cultivars, at the Indicated Arkansas Growing Locations from 2007 to 2010^a

Year	Location						Average NTAT (°C)
	Keiser	Corning	Newport	Pine Tree	Stuttgart	Rohwer	
2007	na	23.9	22.8	na	24.0	24.2	23.7
2008	na	20.7	na	15.5	21.2	19.3	19.2
2009	13.9	na	na	19.3	21.2	20.0	18.6
2010	21.8	na	19.6	22.6	25.8	26.9	23.4

^a Average of ambient temperatures recorded at 30 min intervals during the time of day extending from 8:00 p.m. to 6:00 a.m. na = not a growing location in the indicated year.

from a 32 mm thick clear acrylic sheet, so that no single kernel touched another kernel. A scanned digital image of the kernels was created with an image analysis system (WinSeedle Pro 2005a, Regent Instruments, Sainte-Foy, Quebec, Canada). Prior to measurements, the imaging system was configured to color-classify “chalk” by scanning a brown rice kernel that was considered to be completely chalky as a reference color. For the 100-kernel sets, the imaging system measured and recorded the number of pixels representing the entire kernel area from the scanned images, as well as the number of pixels corresponding to those areas color-classified for chalk. 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. These chalk values were used to investigate the relationship between the presence of chalk and head rice color.

Head Rice Color Analysis

Color of each of the duplicate head rice samples was measured with a colorimeter (Colorflex EZ 45°/0°, Hunterlab, Reston, VA, U.S.A.). Initial color measurements were taken with chalky kernels included. The L^* (black to white) and b^* (blue to yellow) color indices were measured simultaneously. Approximately 35 g of each sample was placed in a 6 cm diameter clear plastic sample cup and centered over a 3 cm sample port. The illuminant and observer settings were D65 and 10°, respectively. An opaque sample cover was placed over each sample prior to measurement to block ambient light, thereby reducing analytical variability. After the first color measurement was taken, the sample cup was rotated 90° and a second measurement was performed. An average of the two readings for each color index (L^* and b^*) was recorded for each duplicate sample.

Because chalk, which manifests as bright, opaque white regions of the kernel, has been shown to increase with increasing NTATs (Ambardekar et al 2011), the authors hypothesized that whiteness values would increase correspondingly because of increasing chalk levels. However, prior to conducting the analysis, it was not known to what degree varying levels of chalk might impact L^* values. Therefore, after the color measurements described in the previous paragraph were taken, a subset of head rice samples was reevaluated for color after chalky kernels were removed, to better understand this relationship. Three long-grain cultivars, Cypress, LaGrue, and XL723, were selected because of their reported susceptibility to the detrimental effects of elevated NTATs, specifically with regard to chalk formation. For each cultivar, samples from two harvest years (2009 and 2010) and four Arkansas locations (Keiser, Pine Tree, Rohwer, and Stuttgart) were selected to encompass a broad range of NTATs. Samples were limited to those with “optimal” HMCs of approximately 19–22% (wb), resulting in a total of 78 samples. Chalky kernels, those with approximately 10% or more of the total area appearing opaque white in color by visual observation, were removed from each head rice sample. The 10% limit was arbitrarily set to minimize the amount of chalk present in a sample and thus to evaluate the effect of NTAT on kernel color exclusive of visible chalk presence. After removal of the chalky kernels,

the remaining head rice was analyzed for L^* values as described previously.

Statistical Analysis

Head rice whiteness (L^*) and yellowness (b^*) values were plotted against NT_{95} 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). Means were compared with Tukey significance tests at a 5% level of probability to indicate significant differences in L^* and b^* values of head rice samples with and without chalky kernels.

RESULTS AND DISCUSSION

Based on field temperatures recorded throughout the study, harvest years 2007 and 2010 were warmer than 2008 and 2009 (Table I). NTATs measured during the critical grain-filling stages (R6–R8) and averaged across all cultivars were greater in the southernmost growing locations of Stuttgart and Rohwer than in the northern locations.

Analysis of the four-year data set showed that L^* values increased significantly as NT_{95} increased during the R7 and R8 reproductive stages, as illustrated for the R8 stage in Figure 1. Across all years, certain cultivars exhibited greater positive correlations than others (Table II). Medium-grain cultivar Bengal, which has been shown to be fairly resistant to the effects of elevated NTAT (Cooper et al 2008; Ambardekar et al 2011; Lanning et al 2011), showed no significant correlations in any R stage. Jupiter, another relatively resistant cultivar, showed significant but weak correlations during only the R8 stage.

Among long-grains, Cypress and Wells did not exhibit significant correlations between L^* and NT_{95} in the R6 stage but did

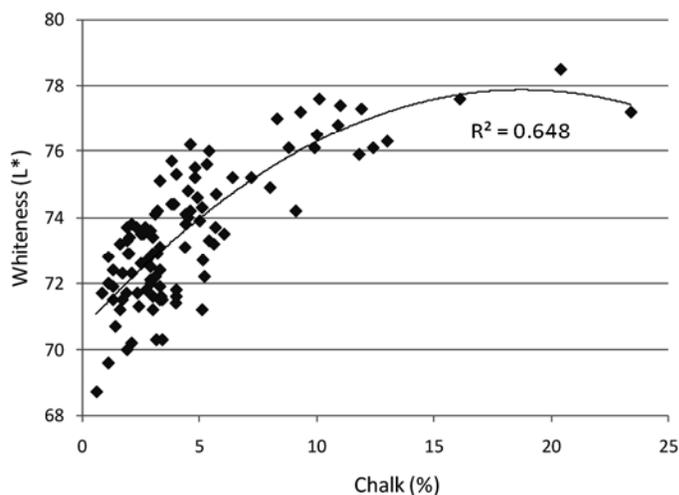


Fig. 2. Effect of chalk on whiteness values of head rice from six cultivars grown across six Arkansas locations from 2007 to 2010. Chalk and L^* values were averaged across a range of harvest moisture contents for each cultivar/location combination.

TABLE II

Coefficients of Determination (R^2) of L^* (Whiteness) and b^* (Yellowness) Values Versus the 95th Percentiles of Nighttime Air Temperature Frequencies During the R6–R8 Reproductive Stages for the Indicated Rice Cultivars Grown Throughout Arkansas from 2007 to 2010^a

Color Parameter	R Stage	Bengal	Jupiter	Cypress	LaGrue	Wells	XL723
L^*	R6	ns	ns	ns	0.525	ns	0.556
	R7	ns	ns	0.545	0.602	0.620	0.487
	R8	ns	0.410	0.700	0.755	0.818	0.739
b^*	R6	ns	ns	ns	ns	ns	ns
	R7	ns	ns	0.368	ns	ns	0.567
	R8	0.469	0.514	0.397	0.269	0.369	0.545

^a ns = not significant ($P > 0.05$).

show significant and relatively strong correlations in R7 and R8. Hybrid cultivar XL723 and pure-line cultivar LaGrue showed significant correlations between L^* and NT_{95} in all three R stages. For each of the long-grain cultivars, correlations strengthened with progression from R7 to R8. These observations paralleled those of Ambardekar et al (2011) and Lanning et al (2011), wherein positive correlations between the presence of chalky kernels and elevated NTATs strengthened from the R7 to R8 stages, and cultivars varied in their degree of susceptibility to chalk formation when exposed to elevated NTATs during these reproductive stages.

Results of the four-year data analysis also indicated a significant ($\alpha \leq 0.05$) positive relationship between whiteness (L^*) and chalk (Fig. 2), suggesting that increases in whiteness with NTATs resulted from corresponding increases in chalk with NTAT (Fig. 3). However, the colorimetric analysis of head rice measured with and without chalky kernels revealed that L^* values did not change significantly with the exclusion of chalky kernels. This observation is illustrated in Figure 4, in which head rice samples of Cypress collected in 2010 from two growing locations (Keiser and Rohwer) were analyzed for whiteness with and without chalky kernels. Whiteness values trended slightly lower with the exclusion of chalky kernels, but the differences were not statistically significant. More importantly, overall whiteness of the sample grown at Rohwer, where NT_{95} (R8) was 32°C , was significantly greater than that of the sample grown at Keiser, where NT_{95} (R8) was only 27°C , even when chalky kernels were excluded from the sample, confirming the trends shown in Figure 1. Similar trends were observed for Cypress and LaGrue cultivars (data not shown). These findings suggested that the correlation between increasing NTAT and increasing whiteness may not be solely related to the visible presence of chalk in kernels. Rather, the authors hypothesize that the effect of NTAT on kernel formation, that is, loose packing of starch granules (Ashida et al 2009), may be manifested not only in the obvious visibly chalky portions of kernels but also in the remaining nonchalky portions of kernels, thus influencing the translucency and whiteness of the kernels. This effect may also impact the starch structure in these nonchalky portions, albeit to a lesser degree than that associated with chalk (Ambardekar et al 2011; Lanning et al 2012).

Trends relating b^* to NT_{95} during the R8 stage were parabolic in nature (Fig. 5), suggesting that yellowness was less apparent at both low and high NTATs than in the intermediate NTAT range. It is notable that the temperature at which yellowness values peaked was approximately 27°C , corresponding closely to the temperature at which chalk formation begins to increase exponentially, as illustrated in Figure 3 (Lanning et al 2011). Furthermore, b^* values generally decreased with increasing chalk in each year of the study (Fig. 6). Across all cultivars and locations, b^* values were significantly lower in 2009, the coolest year of the study; however, b^* values collected in 2008, another cool year, tended to be similar to 2007 and 2010 values ($\alpha > 0.05$), although a much smaller range of values was observed in 2008. Although the results of the current study do not explain the difference in b^* values from 2008 to 2009, they do suggest that although multiple

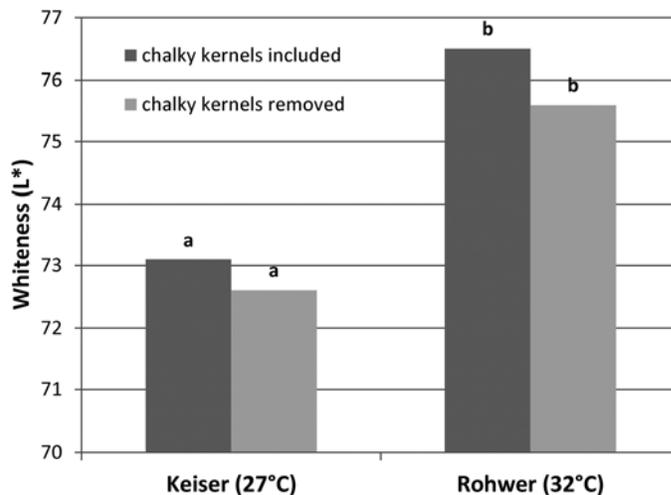


Fig. 4. Whiteness values of Cypress head rice sampled from the indicated growing locations in 2010 and analyzed for whiteness (L^*) with and without chalky kernels. Temperatures noted in the x -axis labels represent 95th percentile temperatures observed during the R8 stage from each respective location. Means with different letters represent different whiteness levels across locations and samples with or without chalky kernels.

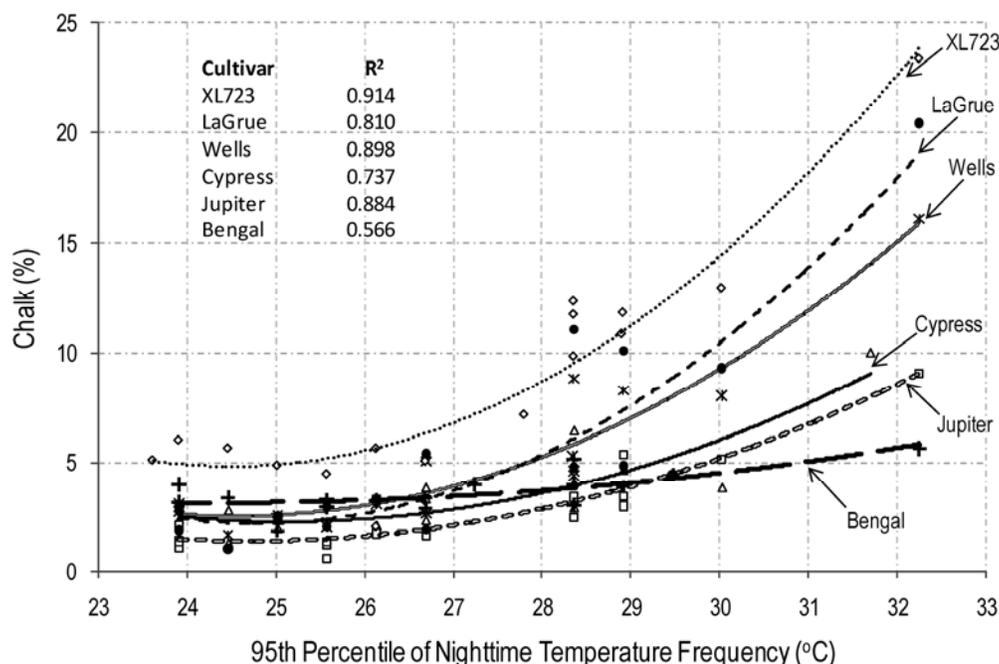


Fig. 3. Relationship of chalk values to 95th percentiles of nighttime air temperature frequencies during the R8 stage for the indicated cultivars grown from 2007 to 2010 (reprinted from Lanning et al 2011).

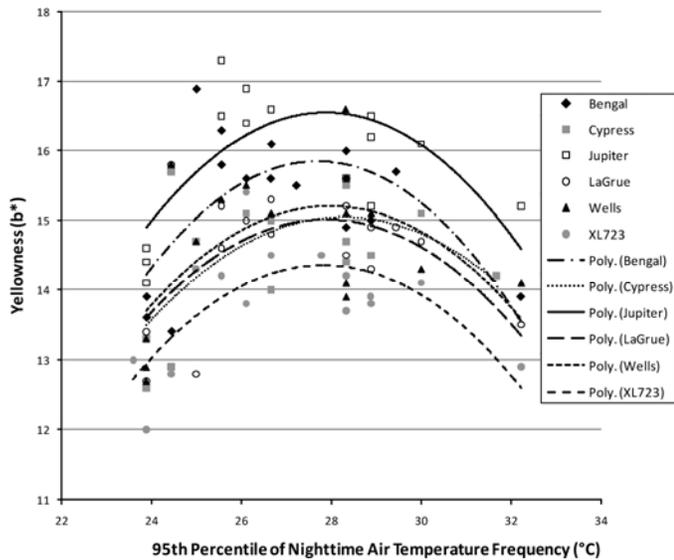


Fig. 5. Relationship of yellowness (b^*) values and 95th percentiles of nighttime air temperature frequencies during the R8 stage for the indicated cultivars grown from 2007 to 2010.

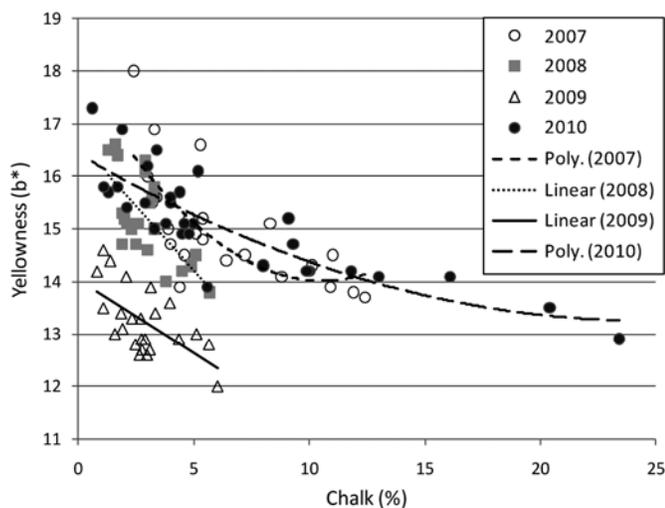


Fig. 6. Effect of chalk on yellowness values of head rice, averaged across six cultivars and six locations for each harvest year, 2007–2010. Chalk and b^* values were averaged across a range of harvest moisture contents for each cultivar/location combination.

environmental factors may influence yellow color formation in milled rice kernels, their effects may be superseded by the impact of elevated NTAT as it relates to kernel formation and chalkiness.

CONCLUSIONS

Elevated NTATs occurring during the critical grain-filling stages of kernel development resulted in increased whiteness values of head rice. Whiteness generally increased, whereas yellowness decreased, with increasing chalkiness. Moreover, kernel whiteness increased with increasing NTAT, even in the absence of chalky kernels. These findings suggested that starch granule organization within the kernel was altered, which could result in compromised structural integrity and changes in starch function-

ality. Cultivars varied in their susceptibility to this response, such that in general, the susceptibility of a cultivar to changes in whiteness resulting from NTAT corresponded to that observed for milling quality and functional properties (Lanning et al 2011, 2012).

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