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Determination of Critical Y-leaf Potassium Concentrations across Reproductive Growth Stages for Direct-Seeded Delay-Flooded Rice

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Abstract

Rice (*Oryza sativa* L.) grain yield can be limited by potassium (K) deficiency on soils low in exchangeable K. Visually diagnosing K deficiency during early reproductive growth is not easily done and the interpretation of traditional tissue analysis is limited to select growth-stages. Our primary focus was to define continuous critical Y-leaf-K concentrations during reproductive growth for the production of maximal grain yield. A secondary objective was to examine Y-leaf sap-K concentration, measured using a handheld device, as a rapid in-field method of monitoring rice plant K nutrition. The Y-leaf is defined as the uppermost fully extended leaf with a visible collar. During reproductive growth, 20 Y-leaves were collected weekly from selected fertilizer-K rates (0 to 150 kg K ha^{-1}) in 13 trials that had suboptimal Mehlich-3 extractable soil-test K and were seeded with either a pure-line or hybrid rice cultivar. For each sample, ten Y-leaves were dried, digested, and K concentration determined using inductively coupled plasma atomic emission spectroscopy. The sap was extracted from ten Y-leaves and the sap-K concentration determined on a handheld Horiba LAQUAtwin B-731 K^+ meter (HMIK, Kyoto, Japan). Rice development was assessed weekly and expressed as growing degree days after R1 stage (DD10R1). The Y-leaf-K concentration increased with increasing fertilizer-K rate and, when evaluated across time, declined for K-sufficient rice, but remained relatively constant for rice that was marginally sufficient or deficient in K. The sap-K concentration trend across time differed among trials, sample times and fertilizer-K rates. The sap-K and leaf-K concentrations were linearly related but the relationship was relatively weak (R^2 = 0.39). The five trials seeded with a hybrid cultivar showed no benefit from fertilizer-K producing mean relative grain yields from 96 to 99%. The relative grain yield of pure-line cultivars ranged from 66 to 99% with significant yield differences measured in five of eight trials. The inconsistency in sap-K

prevented the development of critical sap-K concentrations. The critical Y-leaf-K concentration of pure-line cultivars predicted to produce greater than 95% of maximum yield between the R1 and R2 stage was 16.0 g K kg^{-1}. After the R2 stage, the critical Y-leaf-K concentration gradually declined to 13.7 g K kg^{-1} by the R3 stage but the accuracy of the prediction also declined. The Y-leaf can be used to assess the K nutritional status of pure-line rice cultivars between the R1 and R2 growth stages.

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Chapter 1

Literature Review

Introduction

Rice (*Oryza sativa* L.) is grown on 1.27 million ha in the USA by a select few states including, in order of decreasing acreage, Arkansas, California, Louisiana, Missouri, Mississippi, and Texas (USDA-NASS, 2017a). The USDA-NASS (2017b) showed that in 2016 Arkansas farmers harvested 616,000 ha (1.521 million acres) of rice representing 49% of the USA rice production area. According to periodic surveys of rice growers, the average nitrogen (N) rate and the percentage of the USA acreage receiving fertilizer N has increased to reach a plateau over the last 25 yr, but the average rates and percentage of land area receiving fertilizer phosphorus (P) and potassium (K) continue to increase (Table 1.1). Similar fertilization trends are reported for soybeans [*Glycine max* (L.) Merr.] grown in Arkansas (data not shown, USDA-NASS, 2015). Linear regression of the survey results shows that the K rate applied to rice by Arkansas rice farmers has increased by 1.58 kg K_2O ha^{-1} yr^{-1} since 1991. Despite the trends for increased fertilizer-K rates and the percentage of rice acres to which K is applied, soil-test information indicates soil-K availability is declining in both rice-producing states and non-rice producing states (International Plant Nutrition Institute, 2015). More specifically, soil samples collected following rice and soybean production in Arkansas show the lowest median K concentrations among row crops (DeLong, Slaton, Herron, & Lafex, 2017). For soils cropped to rice and soybean, 30% of the sampled acres had low (61-90 mg K kg^{-1}) or very low (<61 mg K kg^{-1}) soil-test K levels with another 32% of the acres having a medium (91-130 mg K kg^{-1}) soil-test K level.

During the past 30 years, rice and soybean yields have increased at the rate of 58.6 kg ha^{-1} yr^{-1} for rice and 46.7 kg ha^{-1} yr^{-1} for soybean (Fig. 1.1). This corresponds to the removal of an additional 0.12 kg K ha^{-1} yr^{-1} by rice and 0.90 kg K ha^{-1} yr^{-1} by soybean. Although increased

yields result in increased nutrient removal, the increase in annual crop yield does not explain declining soil-test K values following rice and soybean since the rate of crop K removal is lower than the rate of fertilizer-K application. In general, the K harvest index of harvested grain is relatively low in comparison to N and P, as the majority of K taken up by plants remains in the leaves and stems that are usually returned to the field (Dobermann and Fairhurst, 2000). Slaton, Dunn, and Pugh (2004) reported a positive K balance for the eastern one-third of Arkansas where row crops are produced suggesting that soil-test K should theoretically increase in many fields. Assuming that soil-K availability is declining, the occurrence of rice and soybean K deficiency will increase if the trend continues.

Tools that aid farmers in monitoring crop K needs will aid in early identification of K deficiency and allow timely rescue fertilizer applications capable of reducing or preventing potential yield loss. The focus of this literature review is to summarize our knowledge of rice K nutrition and uptake to identify knowledge gaps where additional research is needed with an emphasis on what is known about plant sap analysis as compared to traditional laboratory methods of tissue analysis.

Rice Production Practices

Transplanting and multiple methods of direct seeding are used throughout the world to establish rice stands. In Asia, transplanting is the most common method of stand establishment but it is very labor-intensive (De Datta, 1981). Direct-seeding methods, including water-seeding and dry-seeding, are used predominately in the United States. Drill (80%) or broadcast (15%) seeding of dry rice seed is used for stand establishment on 95% of the Arkansas acreage and water-seeding of dry or pre-sprouted rice accounts for the other 5% (Hardke, 2017). The most

common drill configurations for rice planting include row spacing of 15.24 to 20.32 cm (6 to 8 inches; Wilson, Wamishe, Lorenz, & Hardke, 2018).

Rice is commonly grown following soybean (68%), but rice following rice (continuous rice, 20%), corn (*Zea mays* L.) (4%), and fallow (4%) account for the majority of the other rice rotations (Hardke, 2017). Hardke (2017) summarized that Arkansas rice was produced mainly on silt loam (48%), clay (24%), and clay loam (21%) textured soils using mostly conventional tillage (61%) and stale seedbed (35%) practices. Conservation tillage is thought to be increasing in Arkansas (Wilson et al., 2018) especially when fall weather and soil conditions allow fall tillage and field leveling followed by late winter herbicide application to kill winter vegetation (stale seedbed systems). The stale-seedbed system is especially popular on clayey soils.

Long-grain rice varieties account for about 92% of the Arkansas rice acreage with the remaining 8% planted in medium-grain varieties (USDA-NASS, 2018a). Arkansas rice acreage can also be subdivided into hybrid (43%) vs pure-line rice (57%) cultivars and Clearfield cultivars (hybrid and pure-line cultivars, 45%) that have resistance to imidazolinone herbicides vs conventional lines (55%). Following their introduction in the early 2000s in the United States, the percentage of hybrid rice acres in Arkansas has gradually increased, in part due to their yield advantage (Sha et al., 2014).

Arkansas rice is usually planted from early April through May and harvested in late August through early October (USDA-NASS, 2016). The USDA-NASS (2016) crop progress reports indicate that the five-year (2012-2016) average dates for one-half of the Arkansas rice crop to be planted and harvested commonly occur the first week of May and the middle of September, respectively. However, when weather allows, as in 2016, one-half of the acres may be planted by the second week of April and harvested by early September. The optimal soil

temperature for planting rice is 16°C (60°F) at 10 cm (4 inches) soil depth. Rice seeding rates for pure-line cultivars are 323 seed m^{-2} (30 seed ft^{-2}) when conditions are optimal. The optimal seeding rate for hybrid cultivars is 129 seed m^{-2} (12 seed ft^{-2}) due to their greater vigor and tillering ability and high seed cost compared to pure-line cultivars. Optimal seeding conditions are defined as a conventionally tilled seedbed in good condition (warm temperature and free of clods and crop residue), drill seeded, silt loam texture, and optimum planting date. Seeding rates need to be adjusted by +20% for broadcast seeding, +20% for clay soil, +10% early planting, and +30% late planting (Runsick & Wilson, 2009).

A computerized model called the DD50 (Fahrenheit) or DD10 (Celsius) is free to Arkansas rice producers and predicts crop growth and development for rice grown in the dry-seeded, delayed-flood production system and helps make 26 management decisions (e.g., herbicide application, scouting times, flood times, and fertilizers; Hardke, Wilson, & Norman, 2013). Rice grown with other management systems (e.g., furrow irrigated or water seeded) may alter the accuracy of the model. Model inputs for the DD10 (DD50) include geographic location or county in Arkansas, cultivar name, acreage, and the emergence date. The Arkansas DD10 model uses a modified growing degree day equation, which has a maximum of 17.8 growing degree units d^{-1} (GDU) (32 GDU for DD50). The number of GDU is calculated by entering the daily minimum (with a maximum low temperature of 21°C or 70°F) and maximum (with a maximum high temperature of 34°C or 94°F) temperatures to calculate the daily average and subtract the base temperature (minimum temperature for rice development of 10°C or 50°F, Eq.1 and Eq.2).

[Eq. 1] DD10 = [(daily max temp (°C) + daily min temp (°C)) / 2] – 10

[Eq. 2] DD50 = [(daily max temp (°F) + daily min temp (°F)) / 2] – 50

Rice Growth Stages

Rice growth can be divided into three developmental stages of seedling, vegetative, and reproductive. Counce, Keisling, and Mitchell (2000) and Moldenhauer, Wilson, Counce, and Hardke (2013) provide explanations of rice development, which are summarized in the following text. Seedling development is made up of four stages starting with S0 (unimbibed seed), S1 (coleoptile emergence), S2 (radical emergence), and S3 (prophyll emergence from coleoptile). The prophyll is a leaf sheath with no collar (Moldenhauer et al., 2013). Germination and seedling emergence takes only a few days with an optimum 31°C (87°F) temperature, but can occur within the range of 10 to 42°C (50 to 107°F) when the seed is planted at a depth of 1.3-3.8 cm (0.5-1.5 inches) and has good soil contact.

The vegetative stages are designated as V1, V2…V_N with 'N' representing the number of leaves on the main stem and V_N is the final leaf or flag leaf on the culm (main steam). The V1 stage is the first true leaf after emergence from the prophyll and the V2 to V4 stages are the addition of leaves and altogether is considered the pretillering phase. The start of tillering is V5, and the V stages increase by one with each successive leaf that emerges on the main stem until the flag leaf emerges. According to Counce et al. (2000), a new leaf emerges every 80 to 115 GDU with the main culm producing approximately 15 leaves (Dunand & Saichuk, 2014) during the season. Active tillering occurs after V5 and, for Arkansas growing conditions and cultivars, usually before R0. A vegetative lag stage may occur in some varieties during the period between active tillering and the onset of reproductive growth. Vegetative development takes 24 to 42 d depending on many factors (e.g. moisture, temperature, fertilization, etc…) and the reproductive phases initiate simultaneous with the later V_N stages.

The reproductive phase for most cultivars last about 30 d before the ripening phase begins (Moldenhauer et al., 2013). The R0 stage represents initial panicle development, often referred to as panicle initiation (PI), and is followed by R1 or panicle differentiation (PD), which occurs when parts of the panicle can be differentiated with the naked eye. The R2 stage begins when the sheath starts to swell (early boot stage) and continues until the flag leaf is fully exerted and the collar is visible (late boot stage). The R3 stage begins when the panicle starts to exert from the boot (heading), R4 stage occurs when a minimum of one floret reaches anthesis on the main culm panicle, and R5 stage occurs when a minimum of one caryopsis on the main culm panicle has elongated to the end of the hull (Counce et al., 2000).

The ripening phase progression as described by Moldenhauer et al. (2013) occurs from the R6 to R8 stages and most long-grain varieties take about 35 d to ripen from the R6 stage. During the R6 stage, rice grains contain milk (a white liquid in the kernel) and soft dough (liquid starch starts to firm). The R7 stage represents a minimum of one grain on the main culm panicle has a yellow hull. The R8 stage occurs when a minimum of one grain on the main culm panicle has a brown hull. Collectively the R7 and R8 stages are often referred to as hard dough when rice grains stop filling with milk and the starch dries up causing the grain to harden. The R9 stage is when all grains have a brown hull, at which time the rice is considered mature and ready for harvest. Moisture at the maturity stage is 200 to 220 g H_2O kg^{-1}. The number of days to heading date or 50% heading (R3 stage) is often used as a relative variety maturation guide and occurs when one-half of the panicles have some amount of exertion from the boot. Rice is considered headed when 100% of all panicles have completely emerged from the boot.

Rice Water Management

Water management of rice is very important since it not only prevents water stress, but also integrates all aspects of fertilizer and pest management. According to Hardke (2017), ground water is used to irrigate 74% of Arkansas' rice with the rest irrigated with water from reservoirs (12%) or streams and rivers (14%). To facilitate irrigation, the majority of the rice acres have been leveled, with an estimated 14% being zero-grade, 40% having been precision leveled and the remaining 46% being irrigated with contour levees without precision land leveling. The majority of rice in Arkansas is irrigated with water introduced at the highest elevation point and moves down the field from levee to levee (62%) or multiple inlet (33%) irrigation. Furrow irrigated (2.7%) and intermittent flooding (2.2%) are used on a relatively small amount of the current rice acres. On average, the Arkansas Mississippi Delta Region uses 7601 m^3 irrigation-water ha^{-1} $year^{-1}$ (29.94 acre-inches irrigation-water) to produce flood-irrigated rice (Cooke, Caillavet, & Walker, 1996).

Potassium in Soil

Potassium is relatively immobile in the soil and is present in four forms including the soil solution, exchangeable, non-exchangeable and mineral K pools (Bertsch & Thomas, 1985). Solution K represents the smallest portion of the soil-K, and is easily taken up by plants as the K^+ ion. Soil solution K is susceptible to loss via leaching and runoff because it is not bound to soil colloids by cation exchange forces. The exchangeable K includes K^+ ions that are electrostatically held onto soil cation exchange sites making the size of the exchangeable K pool somewhat dependent on soil cation exchange capacity (CEC). Non-exchangeable K is K ions that are physically trapped between clay lattices and functions to partially restock the exchangeable K that rapidly replenishes the soil solution K. The non-exchangeable K replenishes

exchangeable K but the replacement occurs at a slower rate than the reaction between the solution and exchangeable K pools. Mineral K is not readily available to plants and makes up 90 to 98% of the total K in soil (Weil, 2017).

In Arkansas and other mid-South, rice-producing states, K deficiency of rice is linked to soil texture. Low soil CEC soils, like sandy loams and silt loams, are most likely to be K deficient due to low soil-test (e.g., exchangeable) K, especially when these soils have not been properly fertilized. Clay soils used for rice production in the mid-South USA are normally not K deficient and have high amounts of exchangeable K (Norman, Wilson, & Slaton, 2003).

Patrick, Mikkelsen, and Wells (1985) suggested the anaerobic condition present in flooded soils has more of an impact on N and P availability than K. Flooding soil increases K availability due to displacement of exchangeable K^+ into soil solution by NH_4^+ added as preflood-N fertilizer and by increased concentrations of Fe^{3+} and Mn^{4+} under anaerobic conditions (Norman et al., 2003). The flooded soil condition facilitates the rapid diffusive movement of K ions aiding plant uptake and the equilibration between exchangeable and solution K. Alternating wetting and drying cycles on soils high in 2:1 clay minerals results in high K^+ availability, and during the wetting cycle, the flooded soils show little evidence of K being fixed in an unavailable form (Patrick et al., 1985). High crop yields remove more K from the soil and require higher fertilizer-K rates to prevent soil depletion. Patrick et al. (1985) reported that rice does not always respond to K fertilization because fertilizer K may be rapidly fixed by K-depleted colloids.

Potassium fertilizer recommendations in Arkansas are based on the Mehlich-3 soil test (Slaton et al., 2013). Soil-test K is divided into five levels including very low ($\leq$60 mg K kg^{-1}), low ($\geq$61-90 mg K kg^{-1}), medium ($\geq$91-130 mg K kg^{-1}), optimum (131-175 mg K kg^{-1}) and above

optimum (≥175 mg K kg^{-1}) with fertilizer-K recommendations of 135 (120 lb K_2O $acre^{-1}$), 101 (90 lb K_2O $acre^{-1}$), 67 (60 lb K_2O $acre^{-1}$), 0 and 0 kg K_2O ha^{-1}, respectively (Norman, Slaton, & Roberts, 2013). Slaton, Golden, Norman, Wilson, and DeLong (2009) showed that Mehlich-3 K explained 77 to 81% of the variability in rice tissue-K concentrations at PD and early heading, respectively, and 47 to 63% of the variability in relative yield among soils, suggesting the Mehlich-3 extractant was a reasonably accurate predictor of soil-K availability. Fryer, Slaton, Roberts, Hardke, and Norman (2019) later showed that the recommendations accurately identified crop response to K-fertilization 14 to 20% of the time, but recommendations for soils with optimal soil K availability were 93% accurate. Despite the relatively low accuracy for predicting yield response on soils with suboptimal K availability, Fryer et al. (2019) also reported that Mehlich-3 K was highly correlated with tissue K concentrations (r = 0.85 at R0 and 0.82 at R2-R3) indicating that the soil-test K is a reasonably good assessment of plant-available K.

Rice Uptake of Potassium

Plant uptake of K is equaled or exceeded only by the uptake of N (Yoshida, 1981). According to Barber (1966), K movement to the root system is mainly by diffusion (70-80%) followed by mass flow (10-15%) and root interception (2-5%). In general, plant uptake of K parallels dry matter accumulation. Pugh (2008) reported that the maximum K content of rice occurred at the R3 growth stage with total uptake ranging from 200 to 300 kg K ha^{-1}. Slaton et al. (2009) reported that aboveground K contents >80 kg ha^{-1} at R0 to R1 (PD) and >165 kg K ha^{-1} at R2 to R3 (early heading) were needed for the rice to produce 95% of maximum yield. The highest K uptake rate occurs during vegetative growth and then declines during reproductive growth with no net K uptake after heading is completed (Pugh, 2008). In contrast, before grain

fill, maximal crop growth rate occurs during mid reproductive growth, around the R2 stage. Maschmann, Slaton, Cartwright, and Norman (2010) reported that K uptake by rice was uniformly distributed between the vegetative (V5-R1) and early reproductive growth phases (R1-R3). Maschmann et al. (2010) also reported that the fertilizer-K recovery efficiency by rice receiving 56 and 112 kg K ha^{-1} ranged from 41 to 59% when fertilizer was applied preflood and generally decreased as K application was delayed (32-43% for K applied at R1, 22-36% for K applied at R2).

The peak whole-plant K concentration in rice occurs from late vegetative growth to early reproductive growth (R0) and then gradually declines until heading (R3 stage) indicating that the rate of dry matter accumulation is greater than the rate of K uptake. Straw K content peaks at the R2 to R3 stage and declines as the panicle K content increases during grain development and ripening, showing that K is partitioned from the straw to the panicle as the plant progresses to maturity. The dynamic changes in aboveground rice dry matter accumulation and biomass K concentration mean that the critical K concentration of a plant part likely changes with rice development towards maturity.

Potassium Deficiency of Rice

Potassium is an essential element that functions in photosynthesis, plant water relations, and enzyme activation (Huber, 1985; Mengel, 1985; Suelter, 1985). Potassium-deficient rice is reported to have fewer spikelets $panicle^{-1}$, fewer filled grains, and lower grain weight as compared to K-sufficient rice (Dobermann and Fairhurst, 2000). Maschmann et al. (2010) summarized the literature and reported that K deficiency can cause rice yield losses of up to 50%. In Arkansas, yield losses to K deficiency are generally less than 30% but maybe greater

especially when combined with high incidence and severity of disease (Slaton et al., 2009; Maschmann et al., 2010).

Potassium deficiency symptoms first appear in the older leaves of rice because K is a mobile plant nutrient (Dobermann & Fairhurst, 2000; Norman et al., 2013; Slaton et al., 2011). Deficiency symptoms during vegetative growth include poor vigor and bronzing on older leaves. Symptoms during the reproductive stage may include reduced growth, short droopy and dark green upper leaves, and lower leaves turn yellowish brown first on the leaf tips then the yellowing proceeds along the leaf margins towards the leaf base. The yellowish tissue eventually turns to necrotic spots. As the severity of deficiency increases, the symptoms may appear on the middle and upper leaves. Resistance to some diseases like stem rot (*Magnaporthe salvinii*) and brown leaf spot (*Cochliobolus miyabeanus*) is connected to K deficiency (Huber & Arny, 1985). Slaton et al. (2011) reported that K-deficient rice results in inefficient use of fertilizer-N.

The critical-tissue-K concentration indicating deficiency depends on the growth stage and plant part. The critical concentration and sufficiency ranges cited in the literature are shown in Table 1.2. Unfortunately, not enough information is available to make a comprehensive chart showing the critical K concentration for each stage and plant part. Yoshida (1981) reported the K concentration for K-deficient rice plants to be 15.1 and 12.1 g K kg^{-1} for the upper and lower leaf blades, respectively. The K concentrations in K-deficient plants for the upper and lower leaf sheaths were 9.0 and 5.2 g K kg^{-1}, respectively. It is interesting to note that the K-sufficient concentration for the leaf blades was lower (28.8-29.0 g K kg^{-1}) than that listed for the leaf sheath (33.6-36.8 g K kg^{-1}) opposite of the relationship in K-deficient plants. Slaton et al. (2009) used linear plateau models to establish that the critical minimum whole-plant K concentrations that produced maximal rice yield at R1 stage to be 22.3 g K kg^{-1} and 14.1 g K kg^{-1} at the R2 to

R3 stage which is an 8.2 g K kg^{-1} decline across the 30 d interval from R1 to R3 (0.273 g K kg^{-1} d^{-1}). Relative yields less than 90% of maximum were predicted when whole-plant K concentrations were <17.0 and 10.5 g K kg^{-1} at the R1 and R2-R3 stages, respectively. These results coupled with the models of K concentration across time from Pugh (2008) suggest that the tissue-K concentration between R0 and R3 is predictable, and that continuous critical-K concentrations during reproductive growth can be derived.

Potassium deficiency may be corrected during the season when tissue analysis shows that rice is K deficient. Fertilizer K is typically applied preplant (or preflood) to rice grown in the direct-seeded, delayed-flood system. In-season or post-flood K fertilization is typically performed only after rice shows K deficiency symptoms, which often appear and are noticed between the R0 to R2 development stages (Norman et al., 2013). Fertilizer K applied following the beginning of internode elongation may reduce symptomology, but rice yield response to K fertilization goes down (Dunn et al., 2004; Maschmann et al., 2010). Maschmann et al. (2010) showed rice yield could be significantly increased, but not maximized, by K applied as late as the R2 stage.

Sap Nutrient Analysis

Limited research has been done investigating the use of plant sap-K concentration to diagnose K deficiency of row crops. Sap is the fluid portion of the plants' vacuoles, xylem, and phloem which contains organic and inorganic compounds that are being stored along with traveling through the plant (Dunford, 2015). Analysis of sap extracted from plant petioles has been done on eggplant (*Solanum melongena*), pepper (*Capsicum annuum*), potato (*Solanum tuberosum*), field and greenhouse tomato (*Solanum lycopersicum*), and watermelon (*Citrullus lanatus*) (Hochmuth, 1994); pak choi (*Brassica rapa chinensis*; Gangaiah, Ahmad, Hue, &

Radovich, 2015); tomato (*Solanum lycopersicum*; Rosen, Errebhi, & Wang, 1996); cotton (*Gossypium hirsutum*; Stevens, Rhine, Straatmann, & Dunn, 2016); canola (*Brassica campestris*), chickpea (*Cicer arietinum*), and dwarf sunflower (*Helianthus*; Qian, Schoenau, Greer, Liu, & Shen, 1995); and soybean (Slaton et al., 2017).

Methods for extracting sap from plant tissue and measuring K concentration vary. Stevens et al. (2016) and Gangaiah et al. (2015) used a handheld garlic press while Hochmuth (1994), Rosen et al. (1996), Poehlman (1935), and Pettinger (1931) used a hydraulic press to obtain sap. A creative approach for sap extraction by Burns and Hutsby (1984) was using a homemade, handheld device, like a garlic press, with a 5 mL disposable plastic syringe mounted inside.

The methods of determining sap-K concentrations in the extracted sap also vary among the published research. Burns and Hutsby (1984) measured the sap-K concentration on Merckoquant K test strips (Merck, Darmstadt, Germany). Gangaiah et al. (2015) and Stevens et al. (2016) measured sap-K concentration using a Horiba LAQUAtwin B-731 K^+ meter (Horiba Instruments, Inc., Kyoto, Japan), while Hochmuth (1994) and Rosen et al. (1996) used the original Cardy meter. All of the cited research except for Poehlman (1935) and Pettinger (1931) compared a handheld ion-specific electrode to sap digested in the laboratory and analyzed for K with a spectrophotometer. In general, the results show a linear relationship between sap-K concentrations between the handheld ion meter and laboratory spectrophotometer methods (Table 1.3). Traditional laboratory methods start by digesting dry tissue samples (or the sap itself) followed by analysis using inductively coupled plasma atomic emission spectroscopy (ICP-AES) or atomic absorption spectroscopy (AAS).

Based on the aforementioned literature, plant petioles are the tissue of choice for extracting sap from dicots. Sap is apparently present in a higher volume in petioles than the leaves. Dunn et al. (2004) mentioned that sap could not be extracted from rice leaves. The vast majority of the published research has been performed using dicot plants. Some research evaluating nutrient concentration in plant sap has been done using monocot plants but most of this research was prior to 1950 and the methods are not well suited for rapid in-field testing. Morris and Gerdel (1933) extracted sap from a few different sections of corn plants (e.g., blade, sheath, upper and lower stem, and tassel) and analyzed it using colorimetric and gravimetric procedures. The two methods showed comparable K concentrations in all parts tested (e.g., lower stem had 1.5 mg K mL^{-1} by colorimetric and 1.7 mg K mL^{-1} via gravimetric methods). Krantz, Nelson, and Burkhart (1948) concluded that corn leaf blades were the best part to sample for sap at all growth stages because a uniform sample could be collected by using a certain leaf from each plant (e.g. ear leaf) and sampling could be done nondestructively. The sap extraction and analysis method described by Krantz et al. (1948) was done using potash reagent No. 1 and ethyl alcohol for extraction before the final sap analysis for K concentration was performed using either a visual turbidimetric assessment or colorimetrically using a Klett-Summerson photometer. Pettinger (1931) used a hydraulic press to extract sap from corn with the sap frozen for 1 wk before analysis. Poehlman (1935) used a food processor to chop up whole corn plants followed by pressing the sample in a hydraulic press at a pressure of 34,473.8 kPa (5000 lb in^{-2}) to extract the sap.

Dobermann (2001) mentioned sap analysis of rice as a promising method for assessing the K nutritional status of rice based on unpublished research. The only research with sap-K analysis of rice was reported by Dunn et al. (2004). Dunn et al. (2004) examined the sap-K

concentration, as measured using the Cardy meter, in different parts of the rice plant compared to the K concentration using traditional laboratory methods to determine which plant part was the most appropriate to sample. Dunn et al. (2004) extracted sap using two pieces of angle iron to crush the plants and reported that the basal section of the stem was the only plant part which sap could be extracted from, and also recommended freezing tissue to rupture plant cells to get a greater volume of sap. Dunn et al. (2004) found a positive, linear relationship between sap-K concentration measured on the Cardy meter and traditional lab analysis for the basal section of the rice stem (Table 1.3). Recommendations for use in commercial rice fields were not published from their research.

Plant Sap Issues

Dobermann (2001) reviewed new diagnostic technologies that would aid in crop management and highlighted the use of rapid methods that use fresh plant tissue for real-time K management. Despite the promise of using sap analysis for monitoring row crop K sufficiency, there are a number of obstacles and challenges that require additional research. Some of the challenges and issues of concern for sap nutrient analysis involve the extraction of a sufficient amount of sap for measurement, comparing sap-K concentration among methods of sap extraction, how the time of day influences sap-K concentration, what plant part to extract sap from, the accuracy of quick methods of measuring sap-nutrient concentration, and how storage, dilution with water, and freezing influence sap-K concentration.

The major advantage of sap analysis from fresh plant tissue is the ability to perform the analysis in the field immediately after the sample is collected. However, the collection of large numbers of samples (i.e., different fields), as might be done in a scouting and monitoring program, presents challenges regarding plant tissue and sap collection and storage. Hochmuth

(1994) reported that storing petioles on ice for less than 16 h or freezing petioles for less than 24 h did not significantly change sap-K concentrations. However, Hochmuth's research did not evaluate petiole storage times longer than 16 h. Rosen et al. (1996) confirmed Hochmuth's findings on the lack of an effect of freezing on sap NO_3 concentration, but did not show results. A significant increase in sap-K concentration was observed when the leaves and petioles (i.e., connected) or just petioles were left uncooled in a bag. The increase in sap-K concentration was presumably caused by the leaf wilting and the associated reduction in water content of the leaf blades and petioles (Hochmuth, 1994). Qian et al. (1995) was able to correlate K concentration with both fresh and frozen sap to K concentration of canola sap analyzed using traditional laboratory methods but showed that freezing the plant before extraction resulted in a higher correlation (Table 1.3). They concluded that it did not matter whether the sap was analyzed fresh or frozen, but stated, from a user-friendly perspective, freezing the samples was not necessary since it added time to the process and was practical only if taking a large number of samples. Additionally, they noted that sap could not be extracted from wheat (*Triticum aestivum L.*) without freezing which induces cell lysis and the release of K. Nagarajah (1999) reported that freezing sap significantly increased NO_3-N and K concentrations compared to fresh sap when both nutrients were analyzed using Merck RQflex test strips. Higher K concentrations from frozen sap have also been reported for soybean (Sites and Slaton, unpublished data).

Farneselli, Simonne, Studstill, and Tei (2006) reported that washing the petioles; from muskmelon (*Cucumis melo* L.), bell pepper (*Capsicum annum* L.), and tomato (*Lycopersicon esculentum* Mill) for 30, 60, or 90 s before or after cutting and sap extraction most often reduced the K and NO_3-N concentrations measured on a Cardy meter. The average reduction in K concentration was 19%, which was enough to change the interpretation of the sap-K

concentration. It is interesting to note that the effect of washing was somewhat dependent on plant species. The decision to wash tissue samples may be appropriate only when tissues are dirty or contaminated with foliar applied nutrient solutions that may skew the results.

Timmermans and Van De Ven (2014) claim that conventional tissue testing indicates cumulative nutrient uptake, while sap nutrient analysis provides specific information on the current nutrient availability to the plant. Cassidy (1966) showed that water-soluble constituents like K are mobile nutrients in the plant sap and the need to test sap is necessary to give the true picture of K available to the plant. Sap testing by laboratory techniques to show what is available in the plant may not be necessary for some nutrients due to the availability of nutrient specific handheld devices. One advantage of the hand-held meters, such as those manufactured by Horiba, is that they are capable of performing an in-field measure of sap for selected nutrients eliminating the time-consuming process of sending samples to a laboratory when time-sensitive decisions about nutrient applications need to be made. The Horiba meter costs about $320 (https://www.amazon.com/gp/product/B076F4133R/ref=s9_dcacsd_dcoop_bw_c_x_1_w#feature-bullets-btf) and can be used repeatedly, requiring only minor maintenance and calibration standards (Dobermann, 2001). The greater issue is in extracting sap from fresh tissue. Small amounts of sap can be extracted with a hand-held garlic press, but a large number of samples, as might be encountered in a monitoring program, would require a more user-friendly device for a less strenuous way to extract sap.

The time of day plant sap is collected may have an influence on nutrient concentrations. Nagarajah (1999) showed sap NO_3-N and K concentrations could vary when sampled between 0800 and 1600 h but concluded that samples collected between 0800 and 0930 h prevented this effect. They concluded that plant hydration and salinity effects were likely responsible for the

differences. Panique, Kelling and Schulte (1996) indicated with a small data set that the time of day in which petiole samples were collected did not affect potato sap-K concentrations. Cassidy (1966) also suggested that the time of day should not influence sap-K concentrations because K is water-soluble and plant water content changed by no more than 3% during the day in their experiment.

The handheld Horiba and Cardy meters used for K measurement are stated to be accurate for K concentrations within the range of ±10% of the reading value. The Horiba K meter B-731 can measure the K concentration in a solution of KCl ranging from 39 to 3900 mg K L^{-1} relatively accurately, but displays concentrations from 0 to 8000 mg K L^{-1} (Horiba Scientific, 2012). The new model K-11 which uses the same K ion specific sensor but a different reader has a range of 4 to 9000 mg K L^{-1} and has the same accuracy range as the B-731 model (Horiba Scientific, 2017). The accuracy of measurement is greatest when the K concentrations are low (<3000 mg K L^{-1}) as the error reportedly increases as sap-K concentration increases. Rosen et al. (1996), Taber and Lawson (2007), and Sites and Slaton (unpublished data, 2018) all showed that the sap-K concentration of undiluted sap was quadratically related to the actual sap-K concentration (determined by digestion and ICAP), but the relationship became linear when the sap was diluted. For example, Rosen et al. (1996) showed via standard addition that the recovery of K in undiluted potato petiole sap result was low (69% to 92%), but recovery was near 100% when the sap was diluted. They suggested that sap concentrations above 3000 mg K L^{-1} require dilution to obtain accurate readings that are comparable to laboratory analysis. The literature does not indicate whether sap dilution is needed to make accurate interpretations of plant K sufficiency. If high sap-K concentrations indicate the plant has high or sufficient K concentrations, the reading can be considered an index of sufficiency and no dilution is required.

Summary

Extracting sap from fresh petioles of high-value horticultural crops has become a standard practice. Assessment of the nutritional status of many row crops during the growing season is most often done by traditional tissue analysis. Critical tissue nutrient concentrations are usually published for only one key growth stage, which prevents interpretation of tissue concentrations outside of that growth stage. The use of the Horiba K^+ meter to measure the K concentration in plant sap represents a method that could be done in the field and eliminate the need to send samples to a laboratory for analysis. Such a rapid and inexpensive method could be used to troubleshoot problem fields or monitor a crop continuously during the season. Alternatively, the sap rather than the actual plant tissue could be immediately digested and analyzed in the laboratory eliminating the need to dry, grind and weigh plant tissue.

The ability to quickly and inexpensively monitor a crop's K nutrition might instill greater confidence in growers to follow university fertilization recommendations that are often perceived as too conservative for producing high yields. This literature review i) showed K is needed to maximize rice yield on loamy-textured soils low in K; ii) showed fertilizer-K applied during the season can reduce yield loss from K deficiency; iii) defined K deficiency symptoms and critical tissue concentrations for rice; and iv) highlighted the promise of using plant sap to monitor the nutritional status of rice.

The research focus was to develop continuous critical tissue K concentrations for the reproductive growth stage of rice and evaluate the accuracy of using the Horiba K meter to monitor the K concentration in plant sap. The objectives to be evaluated are:

1. Compare rice tissue K concentrations determined by traditional laboratory procedures (digestion and analysis), the K concentration of plant sap determined with the Horiba K meter, and sap K concentration digested and analyzed by ICP-AES.
2. Develop continuous, critical sap-K and tissue K (traditional analysis) concentrations for rice between the R0 and R4 growth stages by correlating sap and tissue-K concentrations with relative grain yield for 5 to 7 d intervals.

Based on the reviewed literature, the hypothesis for each objective or the anticipated result (when literature to develop a hypothesis is lacking) is:

A significant (P<0.05) linear or quadratic relationship will exist between plant sap-K concentrations measured with the Horiba K^+ meter and tissue or sap digested with traditional laboratory methods (see literature cited in Table 1.3).

Critical concentrations of sap-K and tissue K will be greatest at panicle initiation (R0) and decline at a predictable linear rate from the R0 through R4 development stages.

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Tables and Figure

Table 1.1 Information about rice nitrogen, phosphorus, and potassium fertilization rate and area applied in Arkansas as reported by USDA-NASS (2013a, 2013b).

	Nitrogen		Phosphorus		Potassium		
Year	Acres receiving	Mean rate	Acres receiving	Mean rate	Acres receiving	Mean rate	Planted area
	%	kg N ha^{-1}	%	kg P_2O_5 ha^{-1}	%	kg K_2O ha^{-1}	ha
2013	96	213	76	73	56	95	1521
2006	97	231	68	57	60	86	1406
2000	99	163	44	64	41	70	1420
1992	98	160	12	45	17	66	1400
1991	98	150	10	50	12	58	1300

Table 1.2. Rice published critical tissue K concentrations or sufficiency ranges as based on growth stages and plant section by multiple sources.

Growth stage[a]	Plant section	Critical concentration	Sufficiency range	References
		g K kg^{-1}		
Tillering to PI	Y-leaf	15	--[b]	Dobermann and Fairhurst (2000)
Mid-tillering	Plant	--[b]	15-27	Bell and Kovar (2000)
Max-tillering	Plant	--[b]	12-24	Mills and Jones (1996)
PI	Blade	--[b]	28.8-29.0	Yoshida (1981)
PI	Sheath	--[b]	33.6-36.8	Yoshida (1981)
PI	Plant	--[b]	15-27	Bell and Kovar (2000)
PD	Plant	<17	--[b]	Slaton et al. (2009)
Early heading	Plant	<10.5	--[b]	Slaton et al. (2009)
Flowering	Flag leaf	12	--[b]	Dobermann and Fairhurst (2000)
Maturity	Straw	12	--[b]	Dobermann and Fairhurst (2000)

[a]Growth stage abbreviations: PI, Panicle initiation; PD. Panicle differentiation.
[b] -- means that critical concentration or Sufficiency range not listed by the source.

Table 1.3. Summary of relationships between sap-K concentrations [diluted (DILS) or undiluted, UNDS) as determined using a rapid method intended for field use and traditional (TRAD) laboratory analysis of plant sap.

Crop	Plant Part	Comparison (Y vs X)	Model	R^2	Reference
Grape	Petiole	UNDS vs TRAD	Linear	0.86	Nagarajah (1999)
Tomato	Petiole	UNDS vs TRAD	Quadratic	0.76	Taber and Lawson (2007)
Tomato	Petiole	DILS (1:1) vs TRAD	Linear	0.76	Taber and Lawson (2007)
Tomato	Petiole	DILS (1:4) vs TRAD	Linear	0.96	Taber and Lawson (2007)
Tomato	Leaf	TRAD vs DILS (1:1)	Linear	0.77[a]	Taber and Lawson (2007)
Tomato	Leaf	TRAD vs DILS (1:1)	Linear	0.91[a]	Taber and Lawson (2007)
Tomato	Leaf	TRAD vs DILS (1:1)	Linear	0.90[a]	Taber and Lawson (2007)
Potato	Petiole	UNDS vs TRAD	Quadratic	0.71	Rosen et al. (1996)
Canola	Leaf	UNDS vs TRAD	Linear	0.73	Qian et al. (1995)
Canola[b]	Leaf	UNDS vs TRAD	Linear	0.73	Qian et al. (1995)
Canola	Leaf	UNDS vs TRAD	Linear	0.41	Qian et al. (1995)
Potato	Petiole	TRAD vs UNDS	Linear	0.11	Panique et al. (1996)
Potato	Petiole	TRAD vs DILS (1:9)	Linear	0.61	Panique et al. (1996)
Potato	Petiole	TRAD vs UNDS	Linear	0.33	Panique et al. (1996)
Potato	Petiole	TRAD vs UNDS	Linear	0.44	Panique et al. (1996)
Pak Choi	Petiole, Midrib	DILS (1:5) vs TRAD	Linear	0.65	Gangaiah et al. (2015)
Pak Choi	Petiole, Midrib	DILS (1:5) vs TRAD	Linear	0.69	Gangaiah et al. (2015)
Rice	Basal Stem	UNDS vs TRAD	Linear	0.58	Dunn et al. (2004)
Rice	Basal Stem	UNDS vs TRAD	Linear	0.89	Dunn et al. (2004)
Rice	Basal Stem	UNDS vs TRAD	Linear	0.73	Dunn et al. (2004)

[a]r value
[b]Tissue was frozen before sap extraction

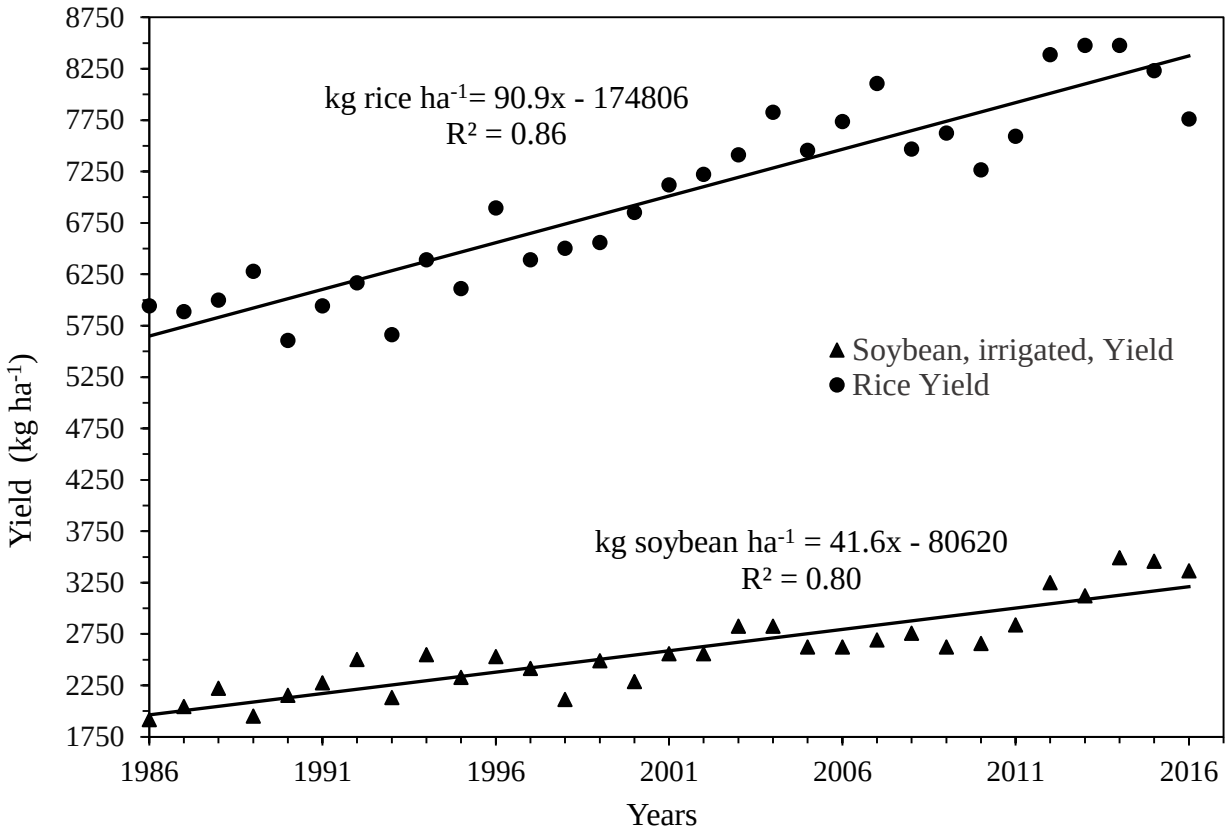

Figure 1.1. Arkansas mean rice and soybean grain yield increase trends across time from 1986 to 2016 as reported by the USDA-NASS (2018b, 2018c).

Chapter 2

Determination of Rice Critical Y-leaf Potassium Concentrations by Traditional Analysis

Abstract

Potassium (K) deficiency symptoms of rice (*Oryza sativa* L.) are difficult to visually diagnose during reproductive growth and critical tissue K concentrations may change across time. Our goal was to define continuous critical Y-leaf-K concentrations during reproductive rice growth for the production of ≥95% rice relative grain yield. Ten Y-leaves were collected weekly during reproductive growth from selected fertilizer-K rates (0-150 kg K ha^{-1}) in 13 trials with Mehlich-3 extractable soil-test K ranging from 32-164 mg K kg $soil^{-1}$ that were seeded with a pure-line (8) or hybrid (5) cultivar. Significant grain yield increases from K fertilization were measured at five of the 13 trials. The K-responsive trials were seeded with a pure-line cultivar and rice receiving no fertilizer K produced 67-90% of the yield produced by rice receiving fertilizer K. Hybrid rice receiving no fertilizer K produced 96-99% of maximum yield. The Y-leaf-K concentrations increased with increasing fertilizer-K rate and tended to decline across time for K-sufficient rice or remained relatively constant across time for rice that was marginally sufficient or deficient in K. Pure-line rice cultivars with Y-leaf-K concentrations above 16.0 g K kg^{-1} between the R1 and R2 stages has sufficient K for maximal yield production. The critical Y-leaf-K concentration declined to about 13.0 g K kg^{-1} between the R2 and R3 stages but was less accurate than before the R2 stage. The Y-leaf-K concentration from pure-line cultivars can be used to assess rice plant K nutritional status between the R1 and R2 growth stages.

Introduction

Potassium deficiency of rice (*Oryza sativa* L.) has become a common malady in many rice-growing areas of the world, including the USA (Cox & Uribe, 1992; Dobermann, Cassman, Mamaril, & Sheehy, 1998; Fryer, Slaton, Roberts, Hardke & Norman, 2019; Regmi et al., 2002). Rice is considered relatively tolerant of K deficiency because it has an extensive fibrous root system (Teo, Beyrouty, Norman, & Gbur, 1995), is frequently grown in flooded soil which enhances soil K availability (Teo, Beyrouty, Norman, & Gbur, 1994), and the grain removes only a small proportion of the plant's aboveground K content (Norman, Wilson, & Slaton, 2003). In Arkansas, Delong, Slaton, Herron and Lafex (2017) reported that 31% of the area cropped to soybean [*Glycine max* (L.) Merr.], the most common crop grown in rotation with rice, had Mehlich-3 soil-test K concentrations considered low (61-90 mg K kg^{-1}) or very low (<61 mg K kg^{-1}) and might benefit from K fertilization when cropped to rice.

Research shows that rice yield increases from K fertilization may range from 187 to 2570 kg ha^{-1} (Fageria, Baligar, Wright, & Carvalho, 1990; Fryer et al., 2019; Regmi et al., 2002; Slaton, Golden, Norman, Wilson, & DeLong, 2009) highlighting the magnitude of potential yield loss and the need for accurate methods of identifying soils that require K fertilization to optimize yield potential. Fryer et al. (2019) reported that rice yield increases to preplant-K fertilization were somewhat unpredictable on soils that were interpreted as having a low level of available-soil K. However, Fryer et al. (2019) and Slaton et al. (2009) both showed the relationship between preplant Mehlich-3 extractable soil K and rice whole-plant K concentration at the R2-R3 (Counce, Keisling, & Mitchell, 2000) development stage was positively correlated ($r > 0.70$) and whole-plant K concentration was a more accurate predictor of rice yield response to preplant-K fertilization than Mehlich-3 extractable soil K.

Maschmann, Slaton, Cartwright, and Norman (2010) reported that the yield of K-deficient rice could be increased by K fertilization as late as the R2 development stage and that fertilizer K applied by the R0 development stage resulted in yields similar to K applied to seedling rice before preflood-N fertilization and flood establishment in the direct-seeded, delayed-flood production system. Singh and Singh (2000) reported that rice yields respond favorably to K fertilizer applied in a split application from preplant through the R1 stage (when internode elongation reaches 12.7 mm). Although the literature contains few examples of how crops respond to mid- and late-season K fertilization, the results of Maschmann et al. (2010) and Singh and Singh (2000) suggest that K can be applied during rice reproductive growth and still produce near maximal or maximal yield. The ability of K-deficient rice to benefit from late-season K fertilization coupled with the inaccuracy of soil-test K to identify soils that respond to K fertilization suggest that alternatives to soil testing for predicting or monitoring the need for rice K fertilization should be evaluated.

The conclusions of Fryer et al. (2019), Maschmann et al. (2010), and Singh and Singh (2000) suggest that the decision to fertilize rice could be made during the growing season using plant tissue analysis. Unfortunately, the literature does not contain consensus recommendations for tissue collection and analysis procedures for assessing the K nutritional status of rice. For example, the Y-leaf (Dobermann & Fairhurst, 2000), top two leaves (Rama Rao & Sekhon 1988), whole plant (Bell & Kovar, 2013; Mills & Jones, 1996; Rama Rao & Sekhon, 1988; Slaton et al., 2009), or straw (Dobermann & Fairhurst, 2000) have been recommended for sampling by different researchers. Each plant part may have a different critical tissue-K concentration or sufficiency range that is limited to one or two growth stages, which highlights the need for more definitive research.

According to Pugh (2008), whole-plant K concentration reaches a maximum near panicle initiation (R0) and then declines at a predictable rate until heading (R3 stage) when aboveground K uptake peaks. Rama Rao and Sekhon (1988) also reported that rice tissue-K concentration declines as the plant progresses through reproductive growth towards maturity. The patterns of cumulative soybean aboveground K uptake (Parvej, Slaton, Purcell, & Roberts, 2016b) and leaflet-K concentration (Parvej, Slaton, Purcell & Roberts, 2016a) across time during reproductive growth shows similarities to that of flood-irrigated rice described by Pugh (2008). Parvej et al. (2016a) showed that leaflet-K concentration of soybean decreased at a predictable and similar rate across time during reproductive growth regardless of maturity group and K fertilization rate. They proposed growth-stage specific critical leaflet-K concentrations for soybean by collecting weekly tissue samples from multiple field trials and correlating soybean relative yield with leaflet-K concentrations at individual growth stages. A similar research approach should work for developing critical tissue-K concentrations across growth stages for rice and other crops.

Accurate identification of crop growth stage, collecting the proper plant tissue, and recognizing that the nutrient critical concentration is dynamic across plant development stages are important for accurate interpretation of plant tissue analysis and correcting in-season nutrient deficiency (Mills & Jones, 1996). The use of a research-based critical nutrient concentration established for a specific crop growth stage at a different growth stage may result in an inaccurate interpretation of tissue analysis and cause a poor nutrient management decision (i.e., negative return on investment). Thus, developing critical nutrient concentrations that change across crop development stages should account for differences in plant development rate among cultivated varieties and allow for the crop development stage to be predicted. Rama Rao and

Sekhon (1988) developed sufficient K concentration ranges at five different rice growth stages using transplanted rice grown in the greenhouse and noted that the strongest growth stage-specific relationships between grain yield and tissue-K concentration occurred near the end of vegetative growth and throughout reproductive growth. Yield and plant tissue-K concentration data from field-grown rice produced with the delayed-flood production system coupled with a meaningful interpretation of time or growth stage are needed to develop continuous critical tissue K concentrations that can be used by analytical labs and agricultural practitioners.

Our research goal was to develop continuous critical tissue-K concentrations by correlating relative rice grain yield with Y-leaf-K concentration from the R1 to R4 development stages. The specific objectives were to examine rice grain yield and Y-leaf-K concentration responses to K fertilization rate and characterize Y-leaf-K concentration across time as affected by fertilizer-K rate and cultivar. Based on the previously mentioned research, we hypothesized that rice relative grain yield would be positively correlated with Y-leaf-K concentration, and the critical Y-leaf-K concentration would decrease at a predictable rate as rice development progressed from the R1 to the R4 growth stage with the maximum Y-leaf-K concentration occurring at R1.

Materials and Methods

Site Description and Treatments

Thirteen field experiments were established during 2018 and 2019 representing short- and long-term K fertilization trials in eastern Arkansas. Trials were conducted at the Pine Tree Research Station (PTRS, Colt, AR) and the Rice Research and Extension Center (RREC, Stuttgart, AR) and each trial will be referred to by the station, year, and letter, if needed, to distinguish among different trials at that station during the same growing season (e.g., PTRS-

18a). The site names and selected soil chemical property information are summarized in Table 2.1. The soil at each site was mapped as a Calhoun silt loam (fine-silty, mixed, active, thermic Typic Glossaqualfs), Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossidalfs), or Dewitt silt loam (fine, smectitic, thermic Typic Albaqualfs). Soybean was the previous crop grown for each site-year except for PTRS-19b and PTRS-19c, which followed rice in rotation. A brief overview of each site-year is provided in the following paragraphs. The soil sampling protocol for each trial consisted of 6 to 8, 2.5-cm diameter soil cores collected from the 0-to 10-cm depth for each composite soil sample. For the long-term trials, one composite sample was collected from every plot between January and March of each year. For short-term trials, a composite sample was collected shortly before fertilizer application and planting from each plot that received no fertilizer K. Soil samples were oven-dried at 65°C for 48 to 72 h, passed through a mechanical grinder and sieve with 2 mm openings. The soil was analyzed for pH in a 1:2 v:v soil to water mixture (Sikora & Kissel, 2014), organic matter by weight loss on ignition (Schulte & Hopkins, 1996), and Mehlich-3 extractable nutrients determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Arcos-160 SOP, Spectro, NJ; Zhang, Hardy, Mylavarapu & Wang, 2014).

The PTRS-18a and PTRS-19a trials are adjacent, long-term K fertilization trials that were established at the PTRS in 2000 and 2001, respectively, and cropped to rice and soybean (Slaton et al., 2017). Five rates of muriate of potash (500 g K kg^{-1}) ranging from 0 to 150 kg K ha^{-1} in 37 kg K ha^{-1} increments are applied preplant each year to the same plots. The trials have been tilled only two times (2004 and 2007) since establishment. Flush or flood irrigation is performed with water from the alluvial aquifer that is high in calcium (Ca) and magnesium (Mg) bicarbonates. Individual plots are 8.0-m wide by 4.9-m long, which accommodates four passes with a 9-row

plot drill having 19-cm wide drill spacings (36 total rows). Soil analysis (Table 2.1) and rice tissue samples were collected from soil receiving 0, 37, 75, and 150 kg K ha^{-1} from four of the eight (PTRS-18a) or nine (PTRS-19a) replicates (Table 2.2). Based on recent yield history, the K rates selected for sampling represent deficient (0 & 37 kg K ha^{-1}), minimally sufficient (75 kg K ha^{-1}) and sufficient (150 kg K ha^{-1}) K nutrition for rice.

The RREC-19 is a long-term K fertilization trial plot that was established in 2007 and rotated annually with soybean (Slaton et al., 2018). Five rates of muriate of potash (500 g K kg^{-1}) ranging from 0 to 150 kg K ha^{-1} in 37 kg K ha^{-1} increments are applied preplant each year to the same plots. The individual plots are 4.6-m wide by 7.6-m long and have not been tilled since 2007. Flush or flood irrigation is performed with reservoir water. Each plot accommodates two passes with an 8-row plot drill (16 total rows of rice) with 19-cm wide row spacings. For the objectives of this study, tissue samples were collected from the 0, 37, and 150 kg K ha^{-1} rates in four of the six replicates on the dates listed in Table 2.2. Based on recent yield history, the three K rates were selected to represent minimally sufficient (0 kg K ha^{-1}), sufficient (37 kg K ha^{-1}) or highly sufficient (150 kg K ha^{-1}) K nutrition for rice. In the nine cropping years before 2017, there were no statistically significant rice yield differences measured among annual-K rates at the RREC location (Slaton et al., 2018).

The remaining ten site-years were single-year trials that provide information from soils that have been managed uniformly across time in regards to K fertilization, and the yield response to K fertilization is unknown beyond what is predicted by soil-K availability (Table 2.1). Each of these trials was drill seeded into a conventionally tilled seedbed. Each short-term trial planted with a pure-line rice cultivar (Diamond or CL 153) contained plots that received one of five K rates (0, 37, 75, 112.5, and 150 kg K ha^{-1}) and four replicates. The trials that were

seeded with a hybrid (Gemini 214 Clearfield) had four replicates with each having rates of 0, 47, 93, and 140 kg K ha^{-1}. The plots for all of the short-term trials at the PTRS were 1.71-m wide (nine rows with 19-cm spacings) by 6.1-to 11.4-m long.

Crop Management

Rice was managed using the drill-seeded, delayed-flood production system, which is used on the majority of rice produced in Arkansas and other mid-South, rice-producing states (Hardke, 2018). The rice was planted at the recommended seeding density rate of 154 to 169 seed m^2 for the hybrid and 382 to 421 seed m^{-2} for both the pure-line cultivars. Emergence dates are listed in Table 2.2. Phosphorus fertilizer (25 kg P ha^{-1} as triple superphosphate, 210 g P kg^{-1}) was applied preplant and Zn fertilizer (1.1 kg Zn ha^{-1}) was applied post-emergence to ensure that these nutrients were not yield-limiting.

Fertilizer nitrogen (N) was broadcast uniformly to each plot area as a single application of urea (460 g N kg^{-1}) treated with N-(n-butyl) thiophosphoric triamide treated (0.89 g NBPT kg^{-1} urea) to supply 115 kg N ha^{-1} for PTRS-18b, PTRS-18d, PTRS-19b, PTRS-19d, PTRS-19f, and RREC-19; 130 kg N ha^{-1} for PTRS-18a, PTRS-18c, PTRS-18e, PTRS-19a, PTRS-19c, PTRS-19e, and PTRS-19g. A 10-cm deep flood was established within 48 h of preflood urea application and maintained until roughly 15 d before the estimated grain harvest date (Table 2.2).

The rice emergence date for each trial was recorded and entered into the DD10 rice management program (e.g., DD50 for °F). The DD10 program calculates growing degree units (GDU) that accumulate during the growing season. The number of GDUs accumulated during a single day is calculated as the daily average temperature (°C) minus the base temperature of 10°C. Daily maximum and minimum temperature thresholds limit the maximum number of GDUs that can be accumulated in a single day to 17.8 (Hardke & Norman, 2018). The program

limits daily maximum temperatures to 34.4°C and daily minimum temperatures cannot fall below 21.1°C.

Plant Sampling and Analysis

Plant samples were collected weekly from the beginning of reproductive growth (R0) through 100% heading (R4 development stage, Counce et al., 2000) representing 6 to 8 sample times across 40 to 45 d. The Y-leaf is the uppermost fully extended leaf with a fully developed collar. At each sample time, ten Y-leaf blades were collected by separating the blade from the leaf sheath at the collar. The Y-leaf samples were collected from an inside row from selected fertilizer-K rates in each trial. Leaf samples were collected from the 0 kg K ha^{-1} rates in PTRS-18b and PTRS-18c; 0 and 140 kg K ha^{-1} rates in PTRS-19g; 0 and 150 kg K ha^{-1} rates in PTRS-19f; 0, 47, and 140 kg K ha^{-1} rates in PTRS-18e, PTRS-19c, and PTRS-19e; 0, 37, and 150 kg K ha^{-1} rates in PTRS-18a, PTRS-18d, PTRS-19b, and PTRS-19d and RREC-19; and 0, 37, 75, and 150 kg K ha^{-1} rates in PTRS-19a. The treatments sampled in each trial were selected based on the anticipated response to fertilizer K and represented treatments expected to produce the lowest, intermediate, and highest yields. The ten leaves were placed in a paper bag, dried in an oven until a constant weight was reached, ground to pass a sieve with 1-mm openings, digested with concentrated HNO_3 and H_2O_2 (Jones & Case, 1990), and the concentrations of K and other nutrients in the digests were determined by ICP-AES.

The rice growth stage was documented for each sample time. Between the R0 (panicle initiation) and R2 (flag leaf collar formation) stages, eight or more stems from each trial were collected, the roots removed, the stems were split longitudinally, and the distance between the visible top and bottom nodes was measured. As plants approached the R2 stage about 3 wk after R1, the sampled plants with a fully emerged flag leaf were counted and expressed as a

percentage of plants at the R2 stage. After the R2 stage, the sampled plants with a partially emerged panicle were counted and expressed as a percentage of plants at the R3 stage. The DD10 computer program uses GDUs to predict rice growth stages including 1.25 cm internode elongation which is an estimate of panicle differentiation and 50% heading with an accuracy of ±2 calendar days using daily temperatures (Hardke & Norman, 2018). Daily high and low temperatures were collected from the nearest weather station [Wynne, AR (Station ID 038052) for trials at PTRS and Stuttgart 9 ESE, AR (Station ID 036920) for the RREC] from the Southern Region Climate Center (https://www.srcc.lsu.edu/). The predicted date of 1.25-cm internode elongation and 50% heading were replaced with actual dates that rice attained these growth stages. The rate of daily internode elongation during the first 15 d after internode movement averaged 3.8 mm d^{-1} in 2018 (n = 9, r^2 = 0.97) and 4.0 mm d^{-1} in 2019 (n = 21, r^2 = 0.80). The time between the R1 to R2 stages was about 3 wk while the 1.5 wk separated the R2 to R3 stages.

Rice Yield

A 3.5-to 11-m^2 area was harvested from the middle five rows in each plot using a small plot combine. A subsample of the harvested grain from each plot was used to determine grain moisture. The grain weights were adjusted to a uniform moisture content of 120 g H_2O kg^{-1} to calculate the final grain yield for statistical analysis. The rice relative grain yield for each K rate treatment within each block of each trial was calculated by dividing the yield in each plot by the highest yielding treatment. This calculation allows for a maximum yield of 100% and places the yields of all trials on a uniform scale of 0 to 100 to account for differences in yield potential among trials as affected by factors such as environment, seeding date, cultivar, or management.

Statistical Analysis

Yield impact from K fertilization

Each trial was a randomized complete block design with data collected from four blocks. Within each trial yield data from all fertilizer-K rates were subjected to ANOVA using the GLIMMIX procedure of SAS (v9.4, SAS Inst., Cary, NC). Significant mean yield differences were compared using LSMEANS ($\alpha = 0.05$)

Y-leaf-K Concentration as Affected by K Rate and Sample Time

The K-concentration data for Y-leaves from selected treatments in each trial were used to examine the trend in Y-leaf-K concentrations across time and determine if Y-leaf-K concentrations could differentiate among fertilizer-K rates and cultivars. Regression was performed on measurements taken between 0 and 640 GDU after the R1 stage (DD10R1) using the GLIMMIX procedure of SAS (v9.4, SAS Inst., Cary, NC) with a gamma distribution and a log transformation of Y-leaf-K concentration data. The Kenward Rogers option was used for computing the denominator degrees of freedom for fixed effects. The DD10R1 time unit was divided by 100 (DD10RH) for SAS to produce estimable coefficients and standard errors. The Y-leaf-K concentrations from replicate observations were regressed across DD10RH allowing for linear and quadratic DD10RH terms with coefficients depending on fertilizer-K rate and cultivar. A final model for each site year was derived by sequentially removing the most complex non-significant model terms ($P>0.10$). The Cooks D and studentized residual (±2.5) statistics were used to identify influential and outlying data points, respectively, which were subsequently removed from the dataset and the model was refit. Pairwise analysis of fertilizer-K rates, cultivars, or sample times was performed using the 95% confidence limits of the prediction at selected points of interest.

Continuous Critical K Concentrations

Continuous, critical Y-leaf-K concentrations were determined using a multiple regression model in GLIMMIX (SAS v9.4, SAS Inst., Cary, NC). The relative yield was regressed across the linear and quadratic terms of cumulative DD10RH and Y-leaf-K concentration plus the linear and quadratic interaction terms involving cumulative DD10RH and Y-leaf-K concentration using a gamma distribution and log transformation of relative yield data. The Kenward Rogers option was used for computing the denominator degrees of freedom for fixed effects. Regression analysis was performed on datasets that included pure-line and hybrid data combined, hybrid cultivar only, and pure-line cultivar only data. The final model for each dataset was derived by sequentially removing the most complex non-significant model terms (P>0.10). The Cooks D and studentized residual (±2.5) statistics were used to identify influential and outlying data points, respectively, which were subsequently removed from the dataset and the model was refit. The final model for pure-line cultivars was used to predict Y-leaf-K concentrations that produced 90 and 95% of the maximum predicted yield. The predicted Y-leaf-K concentrations that produce 90 and 95% of maximum yield were considered the lower and upper boundaries, respectively, of Y-leaf-K concentrations that are considered 'Low'. Dow and Roberts (1982) provided multiple definitions of critical nutrient concentrations, but all the interpretations conveyed the concept of defining nutrient adequacy for producing near-maximal plant growth and yield. Ulrich and Hills (1990) defined critical nutrient concentration "as the concentration at which the growth rate of the plant begins to decline significantly" and usually lies within a transition zone that shows decreasing plant growth as nutrient concentration begins to decrease. For our research, Y-leaf-K concentrations that produce <90% and >95% of the maximum yield were considered ‘Deficient’ and ‘Sufficient’, respectively. The Y-leaf-K concentration

associated with 95% of the maximum yield was defined as the critical concentration and 90 to 95% yield was defined as the critical nutrient range (Low). The predicted Y-leaf-K concentrations associated with 90 and 95% relative yield for each DD10R1 interval were then regressed across cumulative DD10R1. The coefficients from the pure-line model were used to solve for Y-leaf-K concentration at selected time points related to key visual growth stages.

A second regression approach using the REG procedure (SAS v9.4, SAS Inst., Cary, NC), which assumed a normal distribution, was initiated to examine the accuracy of assessing relative yield with Y-leaf-K concentration at selected time intervals. Pure-line cultivar data were sorted into intervals of 100 DD10R1 (e.g., 0-100, 50-150, 100-200, etc… cumulative DD10R1 units) with each successive interval overlapping by 50 DD10R. For each DD10R1 interval, relative yield was regressed across the Y-leaf-K concentrations using a quadratic model, which was simplified to a linear model when the quadratic coefficient was not significant ($P \leq 0.10$). The final model was used to predict the Y-leaf-K concentrations that produce 95% of the maximum yield for the midpoint of each time interval which were regressed across cumulative GDU with a model that included the linear and quadratic time terms.

Results

Rice Yield Response to K Fertilization

Potassium fertilization resulted in a significant rice grain yield increase ($P \leq 0.05$) in five (PTRS-18a, PTRS-18d, PTRS-19a, PTRS-19b, and RREC-19) of eight pure-line trials (Table 2.3) with a sixth site (PTRS-19d) showing consistently higher numerical yields when moderate to high fertilizer-K rates were applied. Pure-line cultivars in the five K-responsive trials produced relative yields that were 66 to 90% of the maximum mean yield with K fertilization resulting in numerical yield increases of 944 to 3229 kg ha^{-1}. The lowest pure-line cultivar grain

yields were produced by rice receiving no fertilizer K and rice receiving the lowest fertilizer-K rate (37 kg K ha^{-1}) produced low to intermediate yields relative to the no-K control and higher fertilizer-K rates. Rice receiving 75 to 150 kg K ha^{-1} produced equal yields that were usually greater than rice receiving no fertilizer K.

Grain yields in the five trials planted to a hybrid cultivar (PTRS-18c, PTRS-18e, PTRS-19c, PTRS-19e, and PTRS-19g) were not significantly influenced by K fertilization. Hybrid rice receiving no fertilizer K produced relative yields ranging from 96 to 99% of the maximum mean yield with only 116 to 404 kg ha^{-1} separating the minimum and maximum yields. The different yield response to K fertilization between hybrid and pure-line cultivars is significant to the overall research objectives of developing dynamic, critical Y-leaf-K concentrations throughout reproductive rice growth.

Y-Leaf-K Concentrations as Affected by K-Rate and Sample Time

The PTRS-18a, PTRS-19a, and RREC-19 trials allow for the comparison of the Y-leaf-K concentration trends across time for pure-line rice grown in experiments that have received the same K rates for 13 or more years. The fertilizer-K treatments in these trials represent the combination of soil-K availability and annual fertilizer-K rates ranging from deficient to low to sufficient with significant grain yield differences among treatments (Table 2.3). The Y-leaf-K concentration trends across time within each trial depended on the fertilizer-K rate (Fig. 2.1 & Table 2.4). For each trial, the intercept values were different among K rates, with the predicted intercept for Y-leaf-K concentration increasing as the fertilizer-K rate increased. In these three trials, the Y-leaf-K concentrations at the R1 stage for rice receiving 150 kg K ha^{-1} yr^{-1} ranged from 19.89 to 31.54 g K kg^{-1} and declined linearly (PTRS-18a & RREC-19) or quadratically (PTRS-19a) with plant development until sampling ended near the R3-R4 stage with predicted

Y-leaf-K concentrations of 8.93 to 13.25 g K kg^{-1}. In contrast, the Y-leaf-K concentration of rice fertilized annually with 0 kg K ha^{-1} followed a quadratic trend across time during reproductive development with the 95% confidence limits indicating the Y-leaf-K concentration was nearly constant from the R1 stage to the R3 stage varying by less than 5.0 g K kg^{-1} during reproductive growth with the maximum numerical concentration occurring 112 to 377 DAR1 and the lowest concentration at the last sample time following the R3 stage (Fig. 2.1 & Appendix 2.1). As K availability to the plant increased the Y-leaf-K concentration also increased with a wider fluctuation between the minimum and maximum Y-leaf-K concentrations during reproductive growth (Appendix 2.1).

Within each of the three long-term trials, the Y-leaf-K concentrations among fertilizer-K rates at the R1 stage were significantly different (Fig. 2.1 & Appendix 2.1). However, by 460 to 480 DD10R1 the predicted Y-leaf-K concentrations were not different among the fertilizer-K rates. This trend suggests that the Y-leaf may be a good indicator of K deficiency during early reproductive growth, prior to R2, but be less effective at diagnosing K deficiency following the R2 stage.

Comparison of the pure-line and hybrid cultivars grown in adjacent areas in the single-year fertilization trials (Fig 2.2 & 2.3) indicates that the general trend described for pure-line rice in the three long-term trials was evident for both cultivar types in the single-year trials. A statistical comparison of the hybrid and pure-line cultivars grown in adjacent areas in the same field and receiving the same (0 kg K ha^{-1}) or similar fertilizer-K rates (140 & 150 kg K ha^{-1}) showed that the two cultivars had similar Y-leaf-K concentrations across time in all but the 0 kg K ha^{-1} fertilizer K-rate comparison of PTRS-19f and PTRS-19g. This information suggests that K availability as influenced by soil-K fertility or fertilizer-K rate had a greater effect on Y-leaf-K

concentration than the phenotypic and genotypic differences between the two cultivars. Thus, Y-leaf-K concentration trend across time does not explain why the hybrid (PTRS-18e, PTRS-19c, PTRS-19e, PTRS-19g) did not respond to fertilizer K while grain yield of the pure-line cultivar (PTRS-18d & PTRS-19b) responded positively at two of the five locations and showed a strong trend at a third location (PTRS-19d).

A comparison of Y-leaf-K concentrations at the R1 stage from rice fertilized with 0 kg K ha^{-1} showed the pure-line and hybrid cultivars had similar Y-leaf-K concentrations in two of the five locations (PTRS-19b & -19c and PTRS-19d & -19e), but the hybrid had greater Y-leaf-K concentrations in the other three adjacent trial sites (Fig. 2.2 & 2.3). Application of 140 or 150 kg K ha^{-1} K rates resulted in similar Y-leaf-K concentrations at the R1 stage in three of the four trials where this comparison was possible (differences only at PTRS-18-d & -18c). However, by the R2 stage, the Y-leaf-K concentrations for like (0 kg K ha^{-1}) or similar (140 or 150 kg K ha^{-1}) K rates were not different. The predicted minimum and maximum Y-leaf-K concentrations during reproductive growth were different for 30 of the 34 fertilizer-K rate, cultivar, and trial combinations (Appendix 2.1). No difference between the minimum and maximum Y-leaf-K concentration occurred only for rice receiving no fertilizer-K at PTRS-18e, PTRS-19b, and PTRS-19c and the hybrid at PTRS-18e fertilized with 47 kg K ha^{-1} (Fig. 2.2 & 2.3).

The R2 stage, which we visually characterized as ≥50% of the plants having a fully emerged flag leaf, occurred at 286 to 470 DD10R1 (1063 to 1172 DD10) in the eight trials planted to a pure-line cultivar (Fig. 2.1-2.3) and 285 to 449 DD10R1 for trials planted with a hybrid cultivar (Fig 2.2. & 2.3), which is 17 to 29 d after the R1 stage. Within each of the eight short-term trials where multiple K-rates were sampled, there were no differences in flag-leaf K concentrations among fertilizer-K rates at and beyond the R2 stage.

Continuous Critical K Concentrations

The relationship between Y-leaf-K concentration, DD10R1, and relative yield was examined by cultivar type (hybrid or pure-line). The model for hybrid only data (n = 289; PTRS-18c, -18e, -19c, -19e, and -19g) was not significant (not shown), which was not surprising since hybrid rice receiving no fertilizer K produced 96 to 99% of the maximum yield within the five trials (Table 2.3). Despite significant differences in Y-leaf-K concentration among fertilizer-K rates and differences across time, the range of relative grain yields for hybrid rice (96 to 100%) among treatments was too narrow to define critical Y-leaf-K concentrations using a 95% yield threshold. The multiple regression model was also examined with both hybrid and pure-line data together, but an examination of the studentized residuals and Cooks D statistic showed more than 10% of the data were flagged as outliers (>±2.5 studentized residuals). Most of the outliers were pure-line cultivars that received no fertilizer K and had low relative yields and the model was abandoned.

For pure-line cultivar data (n = 501; PTRS-18a, -18b, -18d, -19a, -19b, -19d, -19f, and RREC-19), the same modeling process showed that all coefficients included in the initial model were significant in predicting relative grain yield (Table 2.5 & Fig. 2.4). The critical Y-leaf-K concentration was defined as the K concentration that produced 95% of maximum yield. The critical Y-leaf-K concentration of pure-line cultivars was 15.34 g K kg^{-1} at the R1 stage, increased to 16.66 g K kg^{-1} by 220 DD10R1, gradually declined to 16.21 g K kg^{-1} by 355 DD10R1 (50% R2 stage), and declined further to 13.69 g K kg^{-1} at 530 DD10R1, the average time for 50% R3 stage. The range between 90 and 95% relative grain yield was categorized as having low Y-leaf-K concentrations, which were, on average, 2.02 g K kg^{-1} less than the lower

boundary for 95% relative yield. The predicted relative yield decreased by about 5% as Y-leaf-K concentration decreased by 2.0 g K kg^{-1} (Fig. 2.4).

Discussion

The maximum yield increases from K fertilization measured in the five K-responsive, pure-line rice trials (Table 2.3) are comparable to the yield responses documented in the published literature (Fryer et al., 2019; Slaton et al., 2009; Slaton et al., 2010; Ye et al., 2019). Slaton et al. (2010), working mostly with pure-line cultivars, reported the critical Mehlich-3 extractable soil-K concentrations (0-10 cm) needed to produce 90 and 95% of the maximum yields were 80 and 99 mg K kg^{-1}, respectively, suggesting the rice receiving no fertilizer K in 11 of our 13 trials should have responded positively to K fertilization. However, Slaton et al. (2009) noted that 30% of the field sites having Mehlich-3 extractable K <104 mg K kg^{-1} did not respond to K fertilization indicating that soil-test K is not always an accurate predictor of rice yield response to K fertilization. Research by Fryer et al. (2019) reinforced that soil-test K is not always an accurate predictor of rice response to K fertilization. The soil-test K values suggested that no benefit from K fertilization was expected only at trials PTRS-18b and PTRS-18c, which were adjacent and seeded to a pure-line and a hybrid cultivar, respectively (Table 2.1). Reasons explaining why no yield increase from K fertilization was measured in the four trials seeded to a hybrid cultivar and three trials seeded to a pure-line cultivar that had soil-test K < 80 mg K kg^{-1} are not clear but could be due to genotypic traits that influence root growth or K uptake and internal use efficiency (Sanes, Castilhos, Scivittaro, Vahl, & Morais, 2003; Teo et al., 1995; Yang et al., 2003).

The results from the four field sites that had soil-test K values ranging from 45 to 68 mg K kg^{-1} (Table 2.1) with a hybrid cultivar trial established adjacent to a pure-line trial (PTRS-18d

& -18e, PTRS-19b & -19c, PTRS-19d & 19e, and PTRS-19f & -19g) are of notable interest because grain yield of the hybrid cultivar did not benefit from K fertilization (Table 2.3). In contrast, grain yield of the pure-line cultivar grown at two sites (PTRS-18d & PTRS-19b) benefited significantly from K fertilization and yield at a third site (PTRS-19d) benefited numerically. Although these trials were adjacent and had comparable soil-test K values (Table 2.1), they received different fertilizer-K rates and treatments which prohibit direct comparison but is strong evidence suggesting the hybrid cultivars do not respond to K fertilization like pure-line cultivars.

Limited information is available regarding possible differences in yield response to fertilization between pure-line and hybrid cultivars. Nalley, Tack, Barkley, Jagadish, and Brye (2016) reported that hybrid cultivars produced 18 to 20% greater rough rice yields than pure-line cultivars, which is consistent with the yield differences we measured between hybrid and pure-line cultivars in adjacent trials (7-18%). The greater biomass and grain yield produced by hybrid cultivars compared to pure-line cultivars (Mahajan & Chauhan, 2016; Slaton et al., 2010) would seem to increase the demand for and responsiveness to fertilizer K when grown on K-deficient soils as suggested by Doberman and Fairhurst (2000). Hybrids have been shown to respond positively to K fertilization (Ye et al., 2020) and rice genotypes may respond differently to K fertilization (Yang et al., 2003). However, we could not find any literature suggesting consistent differences in response to K between pure-line and hybrid cultivars. The literature does show that hybrid cultivars use soil and fertilizer N more efficiently than the pure-line rice cultivars (Mahajan & Chauhan, 2016; Norman, Roberts, Slaton, & Fulford, 2013) and the more efficient nutrient use is likely because of greater root length, especially under low K conditions (Yang et al., 2003).

Rice root growth response to K deficiency and the mechanism of K use among genotypes is not well understood. Yang et al. (2003) proposed that K deficiency resulted in longer roots in K-efficient cultivars which tended to have lower shoot-K concentrations. Jia, Yang, Feng, and Jilani (2008) reported that severe K deficiency reduced root growth of all genotypes but moderate K deficiency increased the root length of the efficient genotypes which tended to have higher shoot-K concentrations than K-inefficient cultivars. Jia et al. (2008) concluded that changes in root morphology (i.e., more fine roots and greater root surface area under K deficiency) were responsible for the tolerance of K deficiency by K-efficient genotypes. Doberman and Fairhurst (2000) reported that hybrids also have a narrower optimal N:K ratio in the plant than pure-line cultivars and may need more available K due to greater K demand because of the larger above-ground biomass of the hybrid rice plants.

The primary objective of examining the trends of Y-leaf-K concentration across time was to determine if the Y-leaf can be used to assess rice plant-K nutrition. The short- and long-term trials consistently showed that rice, regardless of cultivar, having sufficient available K from the soil, fertilizer, or both to produce maximal yield had high R1 stage Y-leaf-K concentrations that declined as plants progressed through reproductive growth (Fig. 2.1-2.3), the addition of fertilizer K increased Y-leaf-K concentration at the R1 stage, Y-leaf-K concentration was relatively constant from the R1 through the R2 stages in plants that had low to marginally sufficient K availability, and, by the R2 and R3 stages, the Y-leaf-K concentrations among fertilizer-K rates within a trial were similar. Aboveground accumulation of K by flood-irrigated rice peaks by the R3 growth stage (Pugh, 2008), which is similar to the same time that maximal aboveground N accumulation peaks (Guindo, Wells, & Norman, 1994). The declining K concentration of aboveground biomass during reproductive growth can be explained by

decreasing uptake of K by roots and rapid biomass accumulation following the R2 stage resulting in dilution of K in the biomass (Pugh, 2008). Xue et al. (2016) reported that the rice Y-leaf-K concentration increased as the fertilizer-K rate increased, K concentration among leaves was uniform at the jointing stage among fertilizer-K rates, and the range of Y-leaf-K concentrations among fertilizer-K rates was greatest during tillering and jointing and least at the booting and heading stages. Slaton et al. (2010) showed that a hybrid and a pure-line cultivar had similar whole-plant K concentrations for samples collected near the R3 stage.

The trend for the diminishing differences in Y-leaf-K concentration among fertilizer-K rates across time within each trial (Figs. 2.1-2.3) suggests that Y-leaf-K concentrations between the R1 and R2 stages would be more accurate in diagnosing K deficiency than Y-leaf-K concentrations after the R2 stage. The narrow separation among relative yield contours following the R2 stage also supports this hypothesis (Fig. 2.4). The pure-line cultivar data parsed into overlapping intervals of 100 DD10R1 show the coefficient of determination ranged from 0.45 to 0.60 between the R1stage (0 to 100 DD10R1) and 350 to 450 DD10R1 but decreased to 0.29 to 0.43 for all intervals following the 350 to 450 DD10R1 interval (Table 2.6). On average, rice reached 50% R2 stage at 352 (286-470) DD10R1 and 50% R3 stage at 517 (422-586) DD10R1 suggesting that the accuracy of the predicted thresholds is greatest before the R2 stage.

The lower leaf blades or lower leaf sheaths might be better diagnostic tissues to sample than the Y-leaf. Xue et al. (2016) showed the lower leaves had the greatest range in K concentrations among the applied fertilizer-K rates across rice growth stages, but strong correlations also existed between grain yield and Y-leaf blade K concentrations that were numerically comparable to the correlations performed for lower leaf blades, leaf sheaths, or the ratio of the Y-leaf and Y-4 leaf. Xue et al. (2016) concluded that the ratio of leaf-K

concentrations offered an index that was uniform across growth stages and highly correlated with relative rice yield. The disadvantage of the leaf ratio index is the need to sample and analyze the K concentration for two different leaves. Our experience is that by the R2 stage the lower leaves of K-deficient rice plants may be substantially deteriorated from leaf necrosis common to K deficient plants and sheath diseases like stem rot (*Sclerotium oryzae* Catt.) that are known to become more severe under K deficiency (Maschmann et al., 2010). Rama Rao and Sekhon (1988) concluded that the upper two rice leaves were superior to stems for assessing the K nutritional status of rice plants.

Doberman and Fairhurst (2000) reported the critical Y-leaf-K concentration from tillering to panicle initiation (R0) was 15 g K kg^{-1}, which approximates the critical Y-leaf-K concentration defined by our equation to produce 95% of maximum yield at the R1 stage. Doberman and Fairhurst (2000) also suggested that the Y-leaf critical-K concentration at flowering (R3/R4 stages) was 12.0 g K kg^{-1} which compares favorably with our prediction of 11.96 g K kg^{-1} at 530 DD10R1 used to define deficiency (<90% relative yield). Mills and Jones (1996), Slaton et al. (2009), and Bell and Kovar (2013) provided critical-K concentrations and sufficiency ranges for whole plants. Slaton et al. (2009) reported a critical concentration of 17.0 g K kg^{-1} for whole-plant K concentrations at R1 and 13.0 g K kg^{-1} at the R3 stage. Regardless of the plant tissue sampled, our results, as well as the results of Doberman and Fairhurst (2000) and Slaton et al. (2009) agree that the critical plant-K concentrations around the R1 stage are 15.0 to 17.0 g K kg^{-1} and decline to around 13.0 to 14.0 g K kg^{-1} by the R3 stage.

Validating the accuracy of the proposed critical Y-leaf-K concentration thresholds warrants additional research to ensure that other pure-line cultivars behave similarly as the cultivars used in the 13 field trials. The pure-line data were used to provide a preliminary

estimate of threshold accuracy (Fig. 2.5). Across the 640 DD10R1, 31.5% of the pure-line data (64 of 203) having relative yields ≥95% had Y-leaf-K concentrations less than the predicted 95% yield threshold, with the number of errors increasing as DD10R1 increased beyond 300 DD10R1 (Fig. 2.5a). However, only 9% of these data points (19 of 203) had Y-leaf-K concentrations below the 90% of maximum yield threshold. Likewise, 78% of the observations having rice yields < 90% of the maximum yield (128 of 165 observations) were accurately predicted to have Y-leaf-K concentrations below the 90% yield threshold with only 7% (11 of 165) of the observations having Y-leaf-K concentrations greater than the 95% yield threshold (Fig. 2.5c). Of the 131 data points with relative yields between 90 and 95%, the Low K range, 44% (58 of 131) had Y-leaf-K concentrations between the 90 and 95% thresholds, making it the least accurate of the three interpretation groups, which is why it is considered a transition zone between sufficiency and deficiency (Fig. 2.5b).

Conclusions

Pure-line rice cultivars were responsive to K fertilization on silt loam soils low or very low in available K and their Y-leaf-K concentration during reproductive growth was positively correlated with relative grain yield across time. The continuous critical Y-leaf-K concentration for pure-line rice during reproductive growth, from the R1 through the R3 stage, is the novel aspect of our research which provides a means to more accurately interpret Y-leaf-K concentrations that are collected between the R1 and R3 growth stages. The results show that the assessment of the K nutritional status of rice plants is more accurate when performed before the R2 growth stage, defined as 50% of the plants have a fully emerged flag leaf. Between the R1 and R2 development stages, the Y-leaf-K concentration should be above 16 g K kg^{-1} for optimal K nutrition. We also report evidence showing that a hybrid rice cultivar did not respond to K

deficiency and K fertilization the same as the pure-line rice cultivars used in these trials. The lack of response of hybrid rice to K fertilization and the similar trend in Y-leaf-K concentrations across time between hybrid and pure-line cultivars suggests that the vigor imparted by hybridization makes hybrid rice less sensitive to K deficiency and less responsive to fertilization. Roughly one-half of the rice production area in Arkansas is planted to hybrid rice cultivars making cultivar identification an important component for the proper interpretation of Y-leaf-K concentration.

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Tables and Figures

Table 2.1. The site-year, soil series, fertilizer-K rates of long-term trials, and selected soil chemical properties of 13 fertilization trials conducted at the Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR or the Rice Research and Extension Center (RREC) near Stuttgart, AR during 2018 and 2019.

					Mehlich-3 nutrients[c]				
Site-year	Soil series	Fertilizer -K rate	Soil pH[a]	Soil O.M.[b]	P	K	Ca	Mg	Zn
		kg K ha^{-1}	(1:2)	g kg^{-1}	---------- mg kg^{-1} ----------				
PTRS-18a	Calhoun	0	8.1	25.7	42	32	3361	412	9.7
		37	8.1	-	39	51	3318	428	9.1
		150	8.0	-	35	84	2875	397	9.4
PTRS-18b	Calloway	0	6.4	25.6	29	111	1274	214	1.8
PTRS-18c	Calloway	0	6.4	22.8	31	103	1219	197	1.6
PTRS-18d	Calloway	0	7.6	23.1	14	66	2238	324	1.4
PTRS-18e	Calloway	0	7.7	22.7	13	68	1919	316	1.4
PTRS-19a	Calhoun	0	8.1	27.3	37	50	3335	435	7.0
		37	8.3	-	31	47	3305	421	6.2
		75	8.0	-	30	63	3113	430	6.9
		150	8.1	-	30	76	3171	431	6.8
PTRS-19b	Calloway / Calhoun	0	7.5	25.0	12	58	1922	264	1.3
PTRS-19c	Calloway / Calhoun	0	7.5	25.9	10	50	1793	262	1.3
PTRS-19d	Calhoun	0	7.8	23.1	9	46	2142	333	1.5
PTRS-19e	Calhoun	0	7.9	24.2	11	47	2179	345	1.7
PTRS-19f	Calhoun	0	7.9	20.4	20	77	1979	241	6.5
PTRS-19g	Calhoun	0	7.8	20.8	19	65	1707	258	6.9
RREC-19	Dewitt	0	5.5	23.7	50	71	994	147	8.3
		37	5.5	-	44	85	920	136	7.7
		150	5.4	-	45	163	825	121	7.3

[a]Sikora & Kissel (2014)
[b]O.M.= organic matter (Schulte & Hopkins, 1996)
[c]Zhang et al. (2014)

Table 2.2. Agronomic information and leaf sampling dates for 13 K fertilization trials conducted in 2018 and 2019 at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR and the Rice Research and Extension Center (RREC) near Stuttgart, AR.

	Management and growth stage[b] dates					Tissue sample collection dates							
Site-year[a]	Emerged	Flooded	R1	R2	R3	1	2	3	4	5	6	7	8
PTRS-18a	5 May	1 June	20 June	11 July	21 July	20 June	27 June	3 July	10 July	17 July	24 July	1 Aug.	9 Aug.
PTRS-18b	4 May	1 June	20 June	11 July	22 July	20 June	28 June	3 July	10 July	17 July	25 July	-	-
PTRS-18c	4 May	1 June	19 June	11 July	21 July	19 June	28 June	3 July	10 July	17 July	25 July	-	-
PTRS-18d	1 May	31 May	19 June	11 July	23 July	19 June	27 June	3 July	10 July	17 July	25 July	-	-
PTRS-18e	2 May	31 May	13 June	9 July	20 July	19 June	27 June	3 July	10 July	17 July	25 July	2 Aug.	-
PTRS-19a	5 May	4 June	3 July	20 July	29 July	25 June	2 July	9 July	17 July	23 July	30 July	7 Aug.	13 Aug.
PTRS-19b	25 May	19 June	15 July	3 Aug.	13 Aug.	2 July	9 July	16 July	23 July	30 July	7 Aug.	13 Aug.	21 Aug.
PTRS-19c	25 May	19 June	10 July	3 Aug.	15 Aug.	2 July	9 July	16 July	23 July	30 July	7 Aug.	13 Aug.	21 Aug.
PTRS-19d	22 May	13 June	10 July	30 July	10 Aug.	2 July	9 July	16 July	23 July	30 July	7 Aug.	13 Aug.	21 Aug.
PTRS-19e	22 May	13 June	9 July	27 July	7 Aug.	2 July	9 July	16 July	23 July	30 July	7 Aug.	13 Aug.	21 Aug.
PTRS-19f	13 May	5 June	3 July	24 July	3 Aug.	25 June	2 July	9 July	16 July	23 July	30 July	7 Aug.	-
PTRS-19g	13 May	5 June	2 July	20 July	3 Aug.	25 June	2 July	9 July	16 July	23 July	30 July	7 Aug.	-
RREC-19	23 May	20 June	5 July	3 Aug.	10 Aug.	3 July	9 July	17 July	24 July	31 July	6 Aug.	13 Aug.	22 Aug.

[a]Site-year, represents the research station (PTRS or RREC), year (2018 or 2019), and the subsite within the PTRS location for each year (a-g). Trials PTRS-18a, -18b, -18d, -19a, -19b, -19d, & -19e are planted to pure-line cultivar of Diamond with RREC-19 planted to pure-line cultivar of CL153. Trials planted to hybrid cultivar of Gemini CL214 were PTRS-18c, -18e, -19c -19e and -19g.

[b]R1 stage, internode spacing reaches 12.7 mm; R2 stage, 50% of flag leaf collars are visible; R3 stage, 50% of the plants have panicles exerted above the flag leaf collar (Counce et al., 2000).

Table 2.3. Rice grain yield as affected by fertilizer-K rate for 13 trials conducted during 2018 and 2019 at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR or the Rice Research and Extension Center (RREC) near Stuttgart, AR.

		Fertilizer rate (kg K ha^{-1})					
Site-year[a]	Cultivar[b]	0	37/47[c]	75/93	112	150/140	*P* value
		---------- Grain yield (kg ha^{-1})----------					
PTRS-18a	Diamond	8,366c	10,076b	10,588ab	11,062a	11,153a	0.0002
PTRS-18b	Diamond	10,522	10,564	10,027	10,040	10,683	0.2048
PTRS-18c	Gemini	12,168	12,305	12,396	-	12,572	0.2326
PTRS-18d	Diamond	8,760c	9,886b	10,214ab	10,323ab	10,821a	0.0002
PTRS-18e	Gemini	11,715	11,311	11,886	-	11,322	0.3292
PTRS-19a	Diamond	6,228c	7,864b	8,991a	9,440a	9,457a	<0.0001
PTRS-19b	Diamond	7,112b	7,629ab	8,269a	7,850ab	8,506a	0.0263
PTRS-19c	Gemini	8,760	8,885	8,978	-	9,067	0.5281
PTRS-19d	Diamond	7,133	7,066	7,702	7,444	8,279	0.1119
PTRS-19e	Gemini	8,908	9,188	9,135	-	9,303	0.6158
PTRS-19f	Diamond	9,066	8,977	8,922	8,857	9,483	0.3923
PTRS-19g	Gemini	10,321	10,437	10,374	-	10,307	0.9309
RREC-19	CL 153	8,441c	8,715bc	9,097ab	9,385a	9,076ab	0.0374

Note. Within the same site-year (row), means followed by different lowercase letters are statistically different at the 0.05 level.

[a]Site-year, represents the research station (PTRS or RREC), year (2018 or 2019), and the subsite within the PTRS location for each year (a-g). The soil-test K means for each site are shown in Table 2.1.

[b]Diamond and CL153 are pure-line cultivars and Gemini 214 CL is a hybrid cultivar.

[c]The first listed value is the fertilizer-K rate for trials seeded with a pure-line cultivar (Diamond or CL153) and the second listed rate is for trials seeded with a hybrid cultivar (Gemini).

Table 2.4. Regression coefficients and standard errors for predicting rice Y-leaf-K concentrations during reproductive growth as affected by fertilizer-K rate for 13 trials conducted at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR or the Rice Research and Extension Center (RREC) near Stuttgart, AR in 2018 and 2019.

Site-year[a]	Cultivar	Fertilizer -K rate	Intercept[b]	SE	Linear	SE	Quadratic	SE
		kg K ha^{-1}						
PTRS-18a	Diamond	0	2.20	0.035	0.203	0.0266	-0.0267	0.00432
	Diamond	37	2.55	0.030	0.152	0.0229	-0.0276	0.00378
	Diamond	150	3.28	0.030	-0.116	0.0229	0.0012[c]	0.00378
PTRS-18b	Diamond	0	3.05	0.022	0.016[c]	0.0165	-0.0146	0.00264
PTRS-18c	Gemini	0	3.23	0.022	-0.104	0.0160	-0.0004[c]	0.00247
PTRS-18d	Diamond	0	2.09	0.038	0.253	0.0226	-0.0323	0.00343
	Diamond	37	2.32	0.038	0.219	0.0226	-0.0325	0.00343
	Diamond	150	2.79	0.038	0.089	0.0226	-0.0218	0.00343
PTRS-18e	Gemini	0	2.48	0.052	0.095	0.0319	-0.0137	0.00457
	Gemini	47	2.71	0.052	0.061	0.0319	-0.0139	0.00457
	Gemini	140	3.17	0.052	-0.069	0.0319	-0.0032[c]	0.00457
PTRS-19a	Diamond	0	1.89	0.049	0.311	0.0336	-0.0509	0.00576
	Diamond	37	2.36	0.049	0.216	0.0329	-0.0444	0.00560
	Diamond	75	2.62	0.049	0.129	0.0329	-0.0345	0.00560
	Diamond	150	2.99	0.049	-0.009[c]	0.0329	-0.0181	0.00560
PTRS-19b	Diamond	0	2.53	0.029	0.073	0.0162	-0.0168	0.00260
	Diamond	37	2.70	0.029	0.037	0.0162	-0.0146	0.00260
	Diamond	150	3.05	0.029	-0.056	0.0162	-0.0081	0.00260
PTRS-19c	Gemini	0	2.59	0.028	-0.002[c]	0.0166	-0.0042	0.00282
	Gemini	47	2.76	0.028	-0.038	0.0166	-0.0021[c]	0.00282
	Gemini	140	3.11	0.028	-0.131	0.0166	0.0044	0.00282
PTRS-19d	Diamond	0	2.49	0.021	0.133	0.0177	-0.0271	0.00309
	Diamond	37	2.70	0.022	0.069	0.0183	-0.0229	0.00313
	Diamond	150	2.95	0.021	-0.038	0.0175	-0.0106	0.00304

Table 2.4 (cont.)

Site-year[a]	Cultivar	Fertilizer -K rate	Intercept[b]	SE	Linear	SE	Quadratic	SE
		kg K ha^{-1}						
PTRS-19e	Gemini	0	2.60	0.022	0.066	0.0172	-0.0187	0.00295
	Gemini	47	2.80	0.022	0.002[c]	0.0174	-0.0145	0.00295
	Gemini	140	3.06	0.021	-0.105	0.0172	-0.0022[c]	0.00294
PTRS-19f	Diamond	0	2.70	0.022	0.128	0.0142	-0.0294	0.00238
	Diamond	150	3.11	0.022	0.001[c]	0.0142	-0.0180	0.00238
PTRS-19g	Gemini	0	2.88	0.022	0.015	0.0140	-0.0153	0.00228
	Gemini	140	3.15	0.022	-0.091	0.0140	-0.0038	0.00228
RREC-19	CL 153	0	2.81	0.044	0.035	0.0276	-0.0135	0.00378
	CL 153	37	3.10	0.044	-0.046	0.0276	-0.0064	0.00378
	CL 153	150	3.45	0.044	-0.138	0.0285	0.0004[c]	0.00398

[a]Site-year, represents the research station (PTRS or RREC), year (2018 or 2019), and the subsite within the PTRS location for each year (a-g). The three long-term trials include PTRS-18a, PTRS-19a, and RREC-19. The remaining 10 trials were conducted at five sites with a hybrid (Gemini 214 CL) and pure-line (Diamond or CL 153) cultivar planted in adjacent areas (PTRS-18b & PTRS-18c, PTRS-18d & PTRS-18e, PTRS-19b & PTRS-19c, PTRS-19d & PTRS-19e, PTRS-19f & PTRS-19g) and were analyzed
together to comparison of cultivar type (hybrid vs pure-line).
[b]Coefficients derived by first dividing the DD10R1 units by 100 and regression in PROC GLIMMIX using a gamma distribution and log transformation of data. Predicted values can be calculated using the following equation: $e^Y = ax^2 + bx + c$, where Y = sap-K concentration (mg K L^{-1}); x = growing degree units after R1 stage; a = quadratic coefficient, b = linear coefficient, c = intercept; and e = natural exponential function (approximately 2.718281828...).
[c]Coefficients are not significantly different from zero (Pr>0.05).

Table 2.5. Regression coefficients for relative yield as affected by time (DD10R1) and Y-leaf-K concentrations (YLKC) from eight trials seeded with a pure-line cultivar and located at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR and the Rice Research and Extension Center (RREC) near Stuttgart, AR in 2018 and 2019.

Model	Intercept	DD10R1	YLKC	DD10R1 ×YLKC	$DD10R1^2$	$YLKC^2$	$DD10R1^2$ ×$YLKC^2$
Coefficient[a]	3.81[b]	-0.1410	0.0777	0.0072	0.0186	-0.0019	-0.00005
SE	0.049	0.02551	0.00560	0.00176	0.00260	0.00016	0.000013

[a]Regression was performed on DD10R1 units divided by 100 and data were transformed using a gamma distribution. The Y-leaf-K concentration calculated from the regression coefficients must be back-transformed using the exponential function e^x (% relative yield).
[b]Multiple regression equation: e^x (% Relative yield) = 3.81 + (-0.1410×DD10RH) + (0.0777×Leaf K) + (0.0072×DD10RH×Leaf K) + (0.0186×$DD10RH^2$) + (-0.0019×Leaf K^2) + (-0.00005×$DD10RH^2$×Leaf K^2). The relative yield calculated from the regression coefficients must be back-transformed using the exponential function e^x (% relative yield).

Table 2.6. Regression coefficients with standard errors and rice relative yield as predicted by linear or quadratic equations using Y-leaf-K concentrations for data within overlapping 100 growing degree day unit intervals after the R1 growth stage (DD10R1) to produce 95% of maximum relative yield between the R1 and R4 growth stages from eight trials seeded with a pure-line cultivar at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR and the Rice Research and Extension Center (RREC) near Stuttgart, AR in 2018 and 2019.

			Regression coefficients[a]						95% of maximum Yield[b]	
DD10R1	*n*	R^2	Intercept	SE	Linear	SE	Quadratic	SE	Relative Yield	Y-leaf-K
									%	g K kg^{-1}
0-100	63	0.45	53.59	7.162	3.52	0.829	-0.070	0.0223	93.07	16.84
50-150	99	0.61	29.97	6.227	6.08	0.736	-0.136	0.0208	93.02	16.35
100-200	63	0.76	9.68	8.085	8.94	1.078	-0.224	0.0340	93.94	15.26
150-250	87	0.59	12.54	10.091	7.93	1.265	-0.184	0.0390	93.08	16.39
200-300	87	0.59	12.54	10.091	7.93	1.265	-0.184	0.0390	93.08	16.39
250-350	64	0.71	-32.88	15.032	12.75	1.913	-0.309	0.0604	93.71	16.64
300-400	87	0.60	-29.40	16.465	12.50	2.092	-0.306	0.0661	93.35	16.42
350-450	71	0.42	-44.06	29.394	17.11	4.306	-0.525	0.1552	90.58	13.28
400-500	87	0.43	-39.13	29.192	15.77	4.256	-0.457	0.1535	92.07	14.00
450-550	63	0.14	61.60	8.934	2.14	0.655	NS[c]	--	95.00	15.61
500-600	64	0.29	9.54	27.778	11.89	5.131	-0.412	0.2327	90.55	11.03
550-640	75	0.29	5.35	26.022	12.59	4.746	-0.440	0.2134	90.64	11.01

[a]Linear ($y = a + bx$) and quadratic ($y = a + bx + cx^2$) models where y = relative yield (%), x = Y-leaf-K concentration expressed as (g K kg^{-1}), a = intercept coefficient, b = linear slope coefficient, and c = quadratic slope coefficient.
[b]95% of maximum yield calculated by multiplying the predicted maximum yield by 0.95.
[c]Quadratic coefficient was not significant ($P \geq 0.10$) when used in the model.

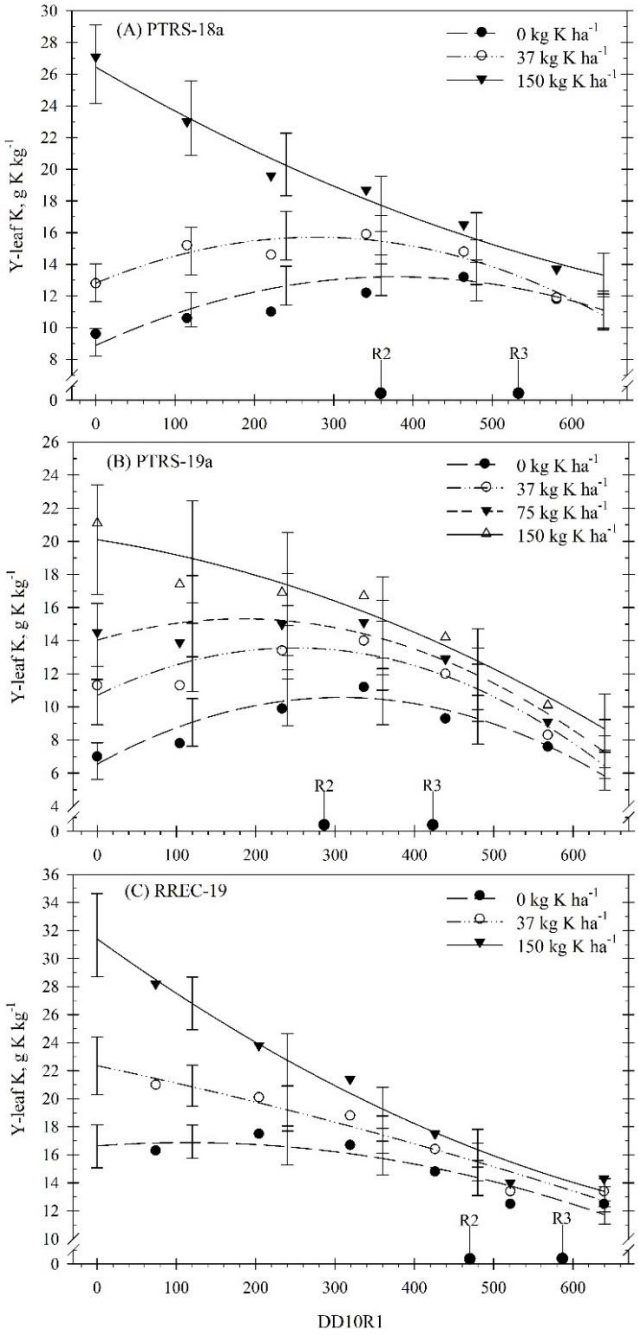

Fig. 2.1. Rice Y-leaf-K concentration beginning with the R1 growth stage through 640 cumulative growing degree units following the R1 stage (DD10R1) for three long-term K fertilization trials planted at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR and the Rice Research and Extension Center (RREC) near Stuttgart, AR during 2018 or 2019 with a pure-line cultivar (PTRS-18a, -19a, and RREC-19, Table 1) as affected by fertilizer K-rate. The error bars at 0, 120, 240, 360, 480 and 640 DD10R1 allow comparison among K rates and across points in time. Regression coefficients are shown in Table 2.4.

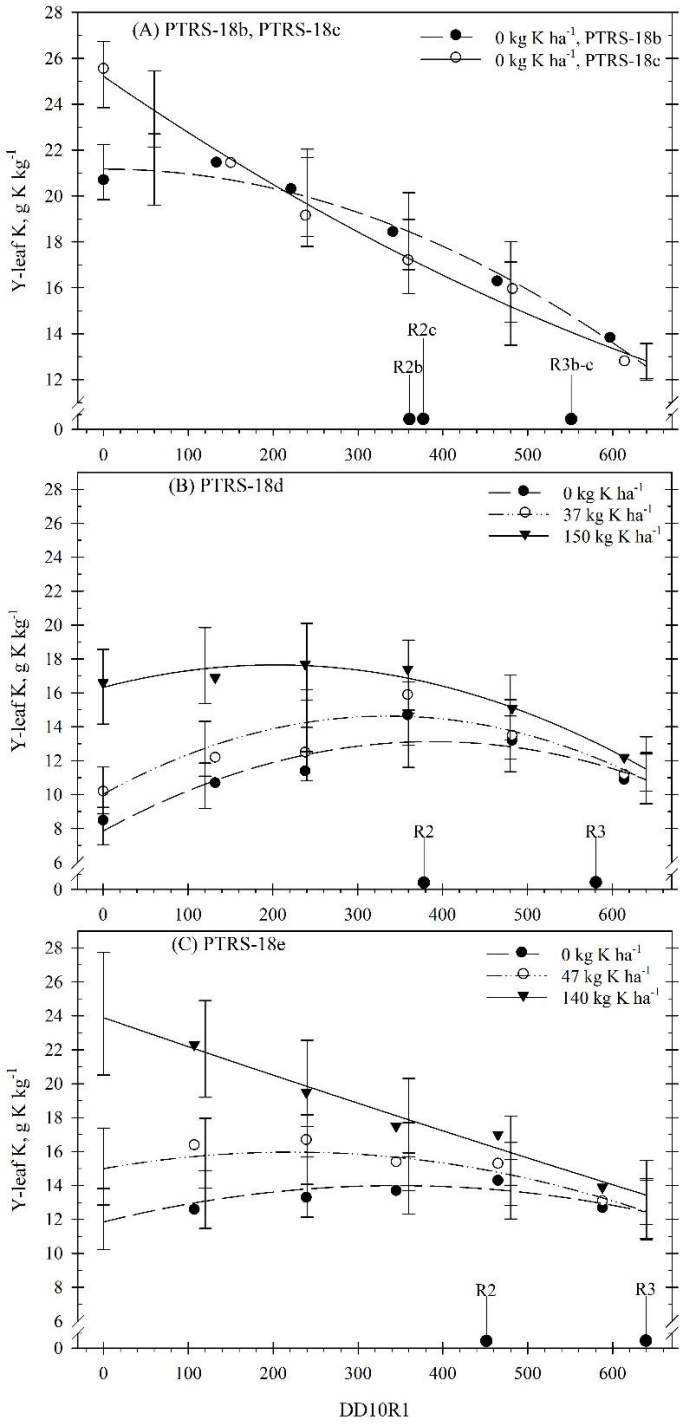

Fig. 2.2. Rice Y-leaf-K concentration beginning with the R1 growth stage through 640 cumulative growing degree units following the R1 stage for three locations having pure-line (PTRS-18b, -18d) and hybrid (PTRS-18c, -18e) cultivars planted at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR during 2018 in adjacent areas as affected by fertilizer-K rates. The error bars at 0, 120, 240, 360, 480 and 640 DD10R1 allow comparison among K rates and across points in time. Regression coefficients are shown in Table 2.4.

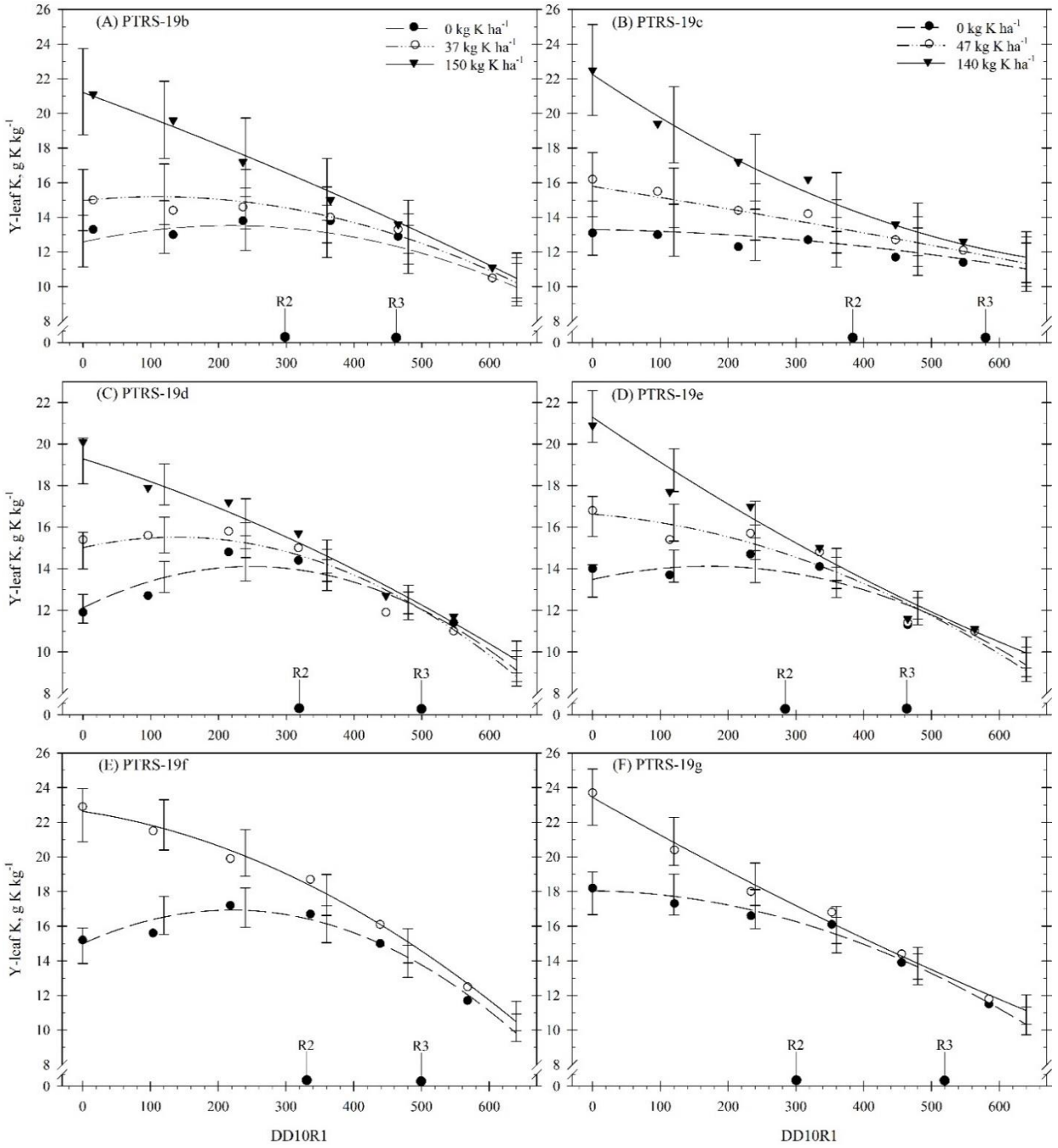

Fig. 2.3. Rice Y-leaf-K concentration beginning with the R1 growth stage through 640 cumulative growing degree units following the R1 stage for three locations having pure-line (PTRS-19b, -19d, -19f) and hybrid (PTRS-19c, -19e, -19g) cultivars planted at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR during 2019 in adjacent areas as affected by fertilizer-K rates. The error bars at 0, 120, 240, 360, 480 and 640 DD10R1 allow comparison among K rates and across points in time. Regression coefficients are shown in Table 2.4.

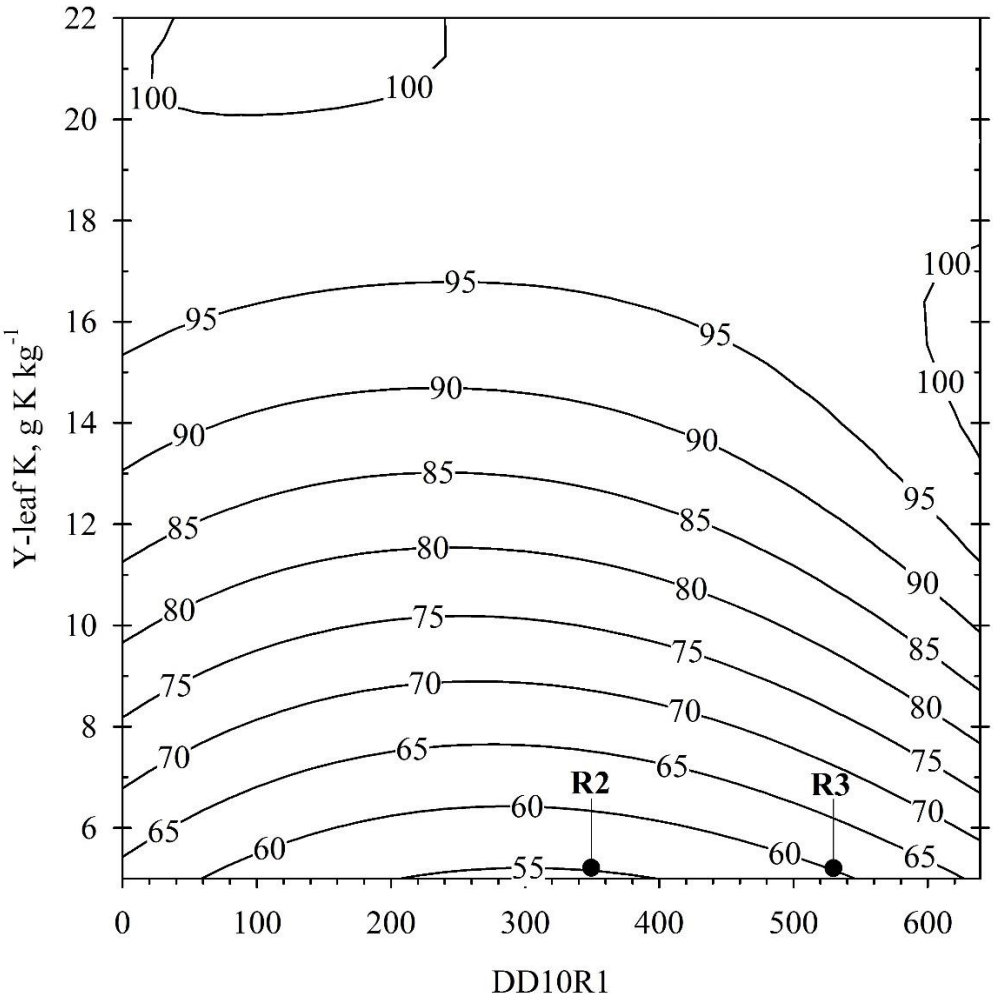

Fig. 2.4. Rice relative grain yield predictions as affected by the cumulative growing degree day units after the R1 stage (DD10R1) and Y-leaf-K concentrations using data from eight trials planted with a pure-line cultivar at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR and the Rice Research and Extension Center (RREC) near Stuttgart, AR during 2018 or 2019 (PTRS-18a, -18b, -18d, -19a, -19b, -19d, -19f, and RREC-19). Model coefficients are listed in Table 2.5.

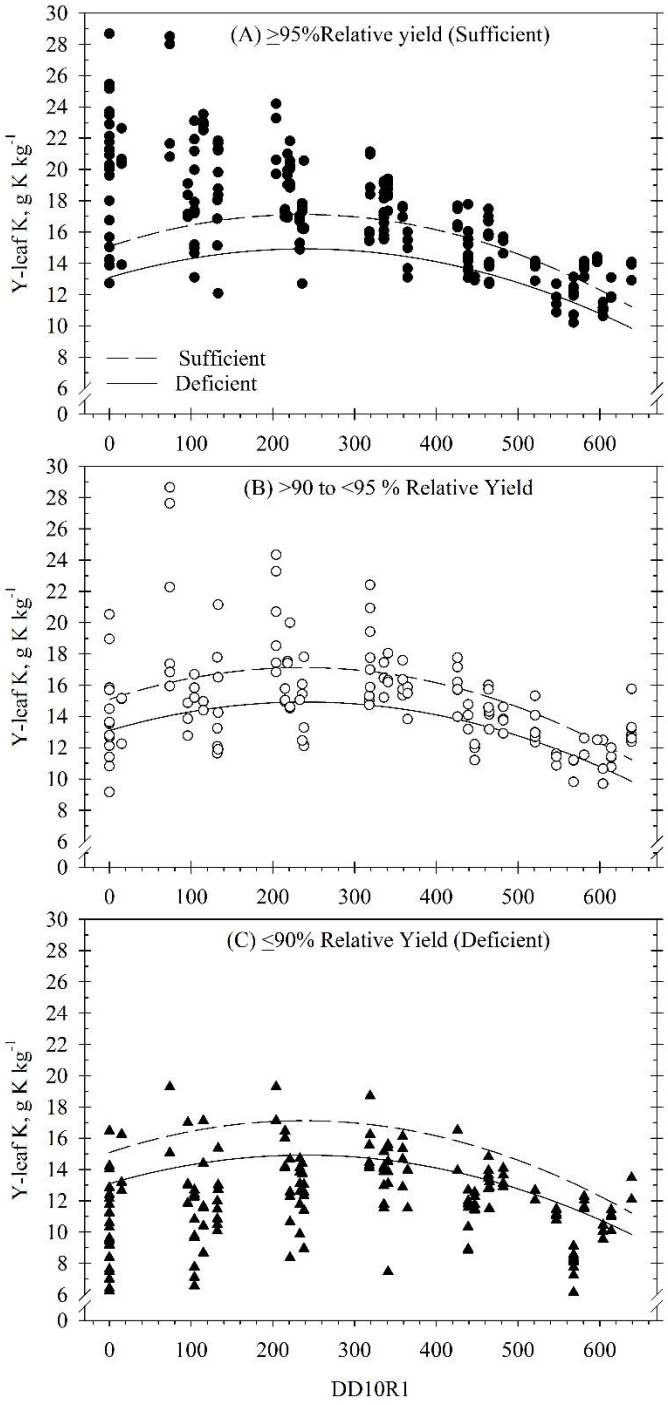

Fig. 2.5. Predicted rice Y-leaf critical tissue-K concentration curves beginning with the R1 growth stage through 640 cumulative growing degree units with actual replicate data points for the definitions of A) sufficient K (>95% relative yield, B) low K (90 to 95% relative yield, and C) deficient K (<90% relative yield). The solid line represents deficient Y-leaf-K concentration ($Y = 13.10 + 0.0152x - 0.000032x^2$) and the dashed line represented sufficient Y-leaf-K concentration ($Y = 15.09+ 0.172x - 0.000036x^2$) as calculated with a quadratic ($y = a + bx + cx^2$) model.

Chapter 3

Comparison of Rice Sap- and Y-leaf-Potassium for Determination of Critical Concentrations

Abstract

A rapid, in-field method of assessing the potassium (K) nutritional status of rice (*Oryza sativa* L.) would help identify fields where K deficiency may limit grain yield. Our focus was to examine the utility of monitoring rice K during reproductive growth by extracting Y-leaf sap and measuring the sap-K concentration using a handheld device compared to the K concentration of the Y-leaf tissue. Twenty rice Y-leaves were collected weekly for 6 to 8 weeks from selected fertilizer-K rates (0-150 kg K ha^{-1}) in six field trials that were seeded with either a pure-line (4) or hybrid (2) cultivar. Ten fresh leaves had sap extracted and analyzed on a Horiba LAQUAtwin B-731 K^+ ion meter (HKIM, Kyoto, Japan). The remaining ten Y-leaves were digested in HNO_3 and analyzed using inductively coupled plasma atomic emission spectroscopy (ICP–AES). The sap- and leaf-K concentrations were linearly and positively correlated but the relationship was relatively weak (R^2 = 0.39). The K concentration of digested sap analyzed by ICP-AES was highly correlated (R^2= 0.87) with the sap-K concentration measured by HKIM but showed the HKIM underestimated the K concentration of undiluted sap. Sap-K concentrations showed no consistent trends across time among trials or treatments but leaf-K concentrations tended to decrease across time when soil- or fertilizer-K availability was high and was usually constant across time when soil- or fertilizer-K availability was low. Extracting Y-leaf sap and measurement of sap-K concentration with the HKIM was not a, accurate method for monitoring rice K nutritional status.

Introduction

In-season potassium (K) monitoring strategies for crop production are needed to help prevent yield losses from K deficiency before plants express deficiency symptoms and suffer irreversible yield loss. Dobermann (2001) suggested that fresh plant tissue-K concentration might be better correlated with plant dry matter and yield than measurements of plant tissue-K concentration based on a dry weight basis. He suggested that the extraction of fresh plant tissue sap is one option for a rapid field measurement of plant K nutrition. Plant sap is defined as the fluid portion of a cell that is made up of inorganic and organic contents that move throughout the plant xylem and phloem and is stored within plant vacuoles (Dunford, 2015). The nutrients in plant sap and their use for assessing plant nutrient status have been the topic of research for nearly 100 years (Poehlman, 1935; Pettinger, 1931). However, method guidelines and critical plant sap nutrient concentrations that define deficient or sufficient levels are available for only a few crop production systems and are used mainly to monitor plant K and NO_3-N nutrition (Hochmuth, Maynard, Vavrina, Hanlon, & Simonne, 2018). Published plant-sap-nutrient monitoring research examining the sap-N and -K concentrations of selected high-value horticultural crops includes trials with eggplant (*Solanum melongena*), pepper (*Capsicum annuum*), pak choi (*Brassica rapa chinensis*), potato (*Solanum tuberosum*), sweet corn (*Zea mays* L.), tomato (*Solanum lycopersicum*), and watermelon (*Citrullus lanatus*) (Gangaiah, Ahmad, Hue, & Radovich, 2015; Hochmuth, Hochmuth, Donley, & Hanlon, 1993; Hochmuth, 1994; Rosen, Errebhi, & Wang, 1996; Taber & Lawson, 2007; White, Tyson, Hanlon, Hochmuth, & Neal, 1996). Research examining the nutritional status of agronomic crops using fresh sap includes cotton (*Gossypium hirsutum*), canola (*Brassica campestris*), rice (*Oryza sativa* L.) and soybean (*Glycine max* L.) (Qian, Schoenau, Greer, Liu, & Shen, 1995; Slaton et al.,

2017a; Stevens, Rhine, Straatmann, & Dunn, 2016). The majority of the published research, regardless of the crop, focuses on comparing fresh sap nutrient concentrations to the tissue nutrient concentration determined using traditional analysis, which includes drying, grinding and digesting plant tissues for analysis in the laboratory (Jones & Case, 1990).

The extraction and analysis of fresh sap have advantages and disadvantages over traditional tissue analysis. Fresh sap is extracted soon after fresh tissue sample collection and can be analyzed immediately using a handheld instrument equipped with an ion-specific electrode or sent to the laboratory (Dobermann, 2001; Hochmuth, 1994). Disadvantages of fresh sap analysis include the lack of information to interpret nutrient concentrations for many plants, handheld instruments available for field use are usually nutrient specific, only small amounts of sap are extracted and sap can be difficult to extract for some plants, handheld instruments may not be accurate (Rosen et al., 1996), and sap concentrations may change as the time between sample collection and extraction increases (Hochmuth, 1994). Proponents of sap analysis suggest that total leaf-K concentration via traditional analysis assesses the total K nutrition of the plant, but sap analysis provides insight on current available nutrient-K status (Timmermans & van de Ven, 2014).

Much of the work performed in the 1980s and 1990s on sap concentrations of K and NO_3-N was performed with the Cardy meter (Hochmuth, 1994; Rosen et al., 1996; Taber & Lawson, 2007; White et al., 1996). The ion-specific Cardy meter manufactured by Horiba Instruments Inc. (Kyoto, Japan) has been replaced by a series of handheld instruments also manufactured by Horiba Instruments Inc. such as the LAQUAtwin B-731 K^+ meter, which has been used in limited research (Gangaiah et al., 2015; Slaton et al., 2017a; Stevens et al., 2016). The published research suggests that K concentrations measured with the Cardy meter and

Horiba handheld instruments are positively related to the sap concentrations measured with more sophisticated laboratory instrumentation (Hochmuth, 1994; Rosen et al., 1996; Taber & Lawson, 2007; White et al., 1996). Literature showing the relationships between sap nutrient concentrations, crop yield and crop yield responsiveness to fertilization is limited to a few publications with limited data (Dunn et al., 2004; Mohr & Tomasiewicz, 2012; Taber, 2006).

Most of the published plant sap research has been performed by sampling the petioles of dicot plants (Hochmuth et al., 1993; Rosen et al., 1996, Taber & Lawson, 2007). Limited research has been performed to examine the utility of sap extraction from monocot plant tissues (Dobermann, 2001; Dunn et al., 2004; White et al., 1996). White et al. (1996) extracted sap from the basal portion of sweet corn leaves and Dunn et al. (2004) extracted sap from mature upper leaves and a 15-cm section from the lower stem of rice. White et al. (1996) showed that sap-K and digested leaf-K concentration declined with plant age and both were able to differentiate among different fertilizer-K rates early in the season but not late in the season. Dunn et al. (2004) reported that sap-K concentration extracted from the basal stem of rice was linearly related with basal stem total-K concentration from digests analyzed using an atomic absorption spectrophotometer, basal stem sap-K or digested stem or total leaf-K concentrations were weakly correlated with rice yield, and sap extraction from rice leaves was very difficult. Unfortunately, neither study provided a sufficient amount of information to truly gauge the success of fresh tissue sap for assessing the K nutritional status of sweet corn or rice. Thus, additional research is needed to assess the utility of sap-K concentration as a quick method for monitoring the K nutrition of monocots.

Rice is grown on about 500,000 ha in Arkansas and K deficiency has been recognized as a yield-limiting factor that is not always accurately predicted by soil testing (Fryer, Slaton,

Roberts, Hardke, & Norman, 2019; Slaton, Golden, Norman, Wilson, & DeLong, 2009). Both Slaton et al. (2009) and Fryer et al. (2019) showed that whole-plant K concentration at the R2 stage was positively correlated with soil-test K and grain yield and a better predictor of relative grain yield than soil-test K. Thus, developing a quick method to assess the K nutritional status of rice during reproductive growth might aid in grower adoption of conservative preplant K recommendations if hidden hunger can be detected by in-season tissue analysis and corrected mid to late season with little or no yield penalty (Maschmann, Slaton, Cartwright, & Norman, 2010).

Our research objectives were to examine i) the relationship between rice Y-leaf sap-K and Y-leaf-K concentration as determined by traditional digestion for tissue analysis, ii) the accuracy of sap-K concentration as determined by the HKIM to that of digested sap analyzed by inductively coupled plasma atomic emission spectroscopy (ICP–AES), iii) the trend of sap-K and leaf-K concentrations across time, and iv) whether the grain yield of rice is related to sap- and leaf-K concentrations across time. Our hypotheses were i) there will be a predictable relationship between sap-K and Y-leaf-K concentrations, ii) a significant (P<0.05) linear or quadratic relationship will exist between sap-K concentrations determined by HKIM and ICP-AES, iii) critical concentrations of sap-K and leaf-K will be greatest at panicle initiation (R0) and decline at a linear rate from the R1 through R4 development stages, and iv) grain yield of rice will be predictable by both sap- and leaf-K concentration regressed across time.

Materials and Methods

Site Description and Treatments

Six field trials were conducted at the Pine Tree Research Station (PTRS, Colt, AR) during 2018 and 2019. These trials will be referred to by the letter designation assigned in Table

3.1. Rice followed soybean in the rotation at all six sites. The soil in each trial was mapped either as a Calhoun silt loam (fine-silty, mixed, active, thermic Typic Glossaqualfs) or a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossidalfs).

Soil chemical properties at each site were assessed by collecting six to eight, 2.5-cm diameter soil cores from the 0-to 10-cm depth of each plot for long-term trials or each plot receiving no fertilizer-K for short-term trials. Long-term trial (Trials A and F) soil samples were collected between January and March of each year while the short-term trials were soil sampled before preplant fertilization and planting. Soil samples were oven-dried at 65°C for 48 to 72 h, ground in a mechanical grinder and passed through a sieve with 2-mm openings. Soil analysis included water pH in a 1:2 v:v soil-to-water mixture (Sikora & Kissel, 2014), organic matter by weight loss on ignition (Schulte & Hopkins, 1996), and Mehlich-3 extractable nutrients analyzed by ICP-AES (Arcos-160 SOP, Spectro, NJ; Zhang, Hardy, Mylavarapu, & Wang, 2014).

Trials A and F (35° 7'15.97"N, 90°57'29.55"W) are adjacent long-term K fertilization trial areas cropped in a rice and soybean rotation and irrigated with water high in calcium (Ca) and magnesium (Mg) bicarbonates from the alluvial aquifer (Slaton et al., 2017b). Every year five rates of muriate of potash (500 g K kg^{-1}) are applied preplant to the same plots with fertilizer-K rates ranging from 0 to 150 kg K ha^{-1} in 37 kg K ha^{-1} increments. These plots have been tilled only twice (2004 and 2007) since establishment in 2000 and 2001, respectively. Trials were planted with a 9-row plot drill having 19-cm wide drill spacings with four drill passes to make a plot 8.0-m wide by 4.9-m long (36 total rows). Three or four K rates in trial A (0, 37, and 150 kg K ha^{-1}) or trial F (0, 37, 75, and 150 kg K ha^{-1}), respectively, were selected for tissue sampling based on recent yield history to represent a range of K nutrition including deficient, minimally sufficient and sufficient K nutrition for rice. Each trial contained four replicates.

Single-year trials comprised the remaining four site-years and supplied information from soils that have had consistent management in terms of K fertilization across time. The Mehlich-3 extractable K was considered low (61-90 mg K kg^{-1}) or medium (91-130 mg K kg^{-1}) by the University of Arkansas recommendations (Roberts, Slaton, Wilson, & Norman, 2018). Trials B and D were drill seeded into a conventionally tilled seedbed with a 9-row plot drill having 19-cm row spacing. Individual plots were 1.7-m wide and 5.7-m long. The pure-line cultivar Diamond was planted (382 to 421 seed m^{-2}) in Trials B and D and included five K rates (0, 37, 75, 112, and 150 kg K ha^{-1}) and four replicates. Trials C and E were seeded with 154 seed m^{-2} of the hybrid rice cultivar Gemini 214 Clearfield (RiceTec Inc., Alvin, TX) into conventionally tilled seedbeds and included rates of 0, 47, 93, and 140 kg K ha^{-1} and four replicates. The pure-line and hybrid cultivar in Trials B and C (35° 6'54.92"N and 90°56'20.64"W) and Trials D and E (35° 3'58.96"N and 90°56'43.00"W) were each planted in adjacent areas in the same field which had similar soil properties (Table 3.1).

Crop Management

The rice production system used in all trials was the drill-seeded, delayed-flood production system outlined by Hardke (2018). Phosphorus (25 kg P ha^{-1}) as triple superphosphate (210 g P kg^{-1}) was applied to each trial preplant and a Zn solution (1.1 kg Zn ha^{-1}) was spray applied to rice foliage post-emergence. Urea fertilizer treated with N-(n-butyl) thiophosphoric triamide (0.89 g NBPT kg^{-1} urea) was broadcast as a single preflood application to each trial to supply 115 kg N ha^{-1} for trials B (N application, 30 May) and D (29 May); 130 kg N ha^{-1} for trials A, C, E, and F (N applications on 29 May, 30 May, 29 May, and 4 June, respectively). A 10-cm deep permanent flood was established within 48 h of urea application and maintained until 15 d before harvest.

The DD10 rice management program (e.g., DD50 for °F) was used to calculate growing degree units (GDU) accumulated during the growing season starting from the date of rice emergence. The number of daily GDUs is calculated by using the average temperature (daily maximum + minimum temperature (°C)/2) and subtracting the 10°C base temperature (Hardke and Norman, 2018). The DD10 GDU calculation has a daily maximum accumulation of 17.8 GDU due to upper thresholds for maximum and minimum daily temperatures of 34.4°C and 21.1°C, respectively. Temperature data after rice emergence was obtained from the nearest weather station [Wynne, AR (Station ID 038052)] used by Southern Region Climate Center (https://www.srcc.lsu.edu/) for daily high and low temperatures.

Plant Sampling and Analysis

The Y-leaf samples were collected weekly from near the start of reproductive growth (R0) through 100% heading (R4 development stage; Counce, Keisling, & Mitchell, 2000) spanning 6 or 7 sample times during a 40 to 45 d interval. The Y-leaf, defined as the uppermost leaf with a visible collar, was collected from 20 plants in each plot by removing the leaf from the sheath at the collar at the sample times listed in Table 3.2. Leaf samples were collected only from interior rows. Ten of the leaves were placed in a labeled paper bag for traditional plant analysis in which the leaves were dried in a 65°C forced draft oven, ground to pass a 1-mm sieve, digested with concentrated HNO_3 and H_2O_2 digestion (Jones & Case, 1990), and an ICP-AES was used to determine the concentration of K and other nutrients (Arcos-160 SOP, Spectro, NJ). The ten remaining leaves were placed in a second, labeled paper (2018) or plastic (2019) bag and stored in an ice-filled cooler (but not in contact with the ice), and transported to a nearby lab for sap extraction. The leaves were cut into 1-cm long pieces, placed in a manufactured sap press mounted on a frame that fits into a truck hitch, and the sap was extracted into a 14.2 ml

vial. The amount of sap extracted ranged from 0.5 to 2.0 ml. The extracted sap was stored on ice or refrigerated for 4 to 48 h before a subsample was analyzed on a calibrated HKIM. The HKIM was calibrated with the two standards sold with the instrument (150 and 2000 mg K L^{-1}) following the instructions in the manual (Horiba, 2012; http://www.horiba.com/fileadmin/uploads/Affiliates/hor/Documents/Application/Water_Quality/Documents/GZ0000297061_IM_E_B-731.pdf). The vial containing fresh sap was allowed to equilibrate to room temperature (21-23°C), mixed, and a 0.5 to 0.75 ml aliquot of sap was placed on the HKIM sensor with a disposable pipette. The HKIM sensor was rinsed with deionized water and blotted dry between samples. The accuracy of the HKIM was checked with six standards having K concentrations from 500 to 8000 mg K L^{-1} made from reagent grade KCl. The vials of sap were frozen for storage and additional analysis.

Fifty of the sap samples collected in 2018, representing a range of sap-K concentrations as determined with the HKIM, were digested to determine the actual concentration of K and other nutrients in the sap using standard lab methods (Table 3.3). Briefly, the frozen sap samples were thawed and mixed on a vortex mixer, a 0.5 ml aliquot of sap was pipetted from the vial into a tared flask, the weight of the aliquot was recorded, and the specific gravity was calculated. The rice sap was digested with concentrated HNO_3 and H_2O_2 (Jones & Case, 1990) and analyzed by ICP-AES to determine the concentration of K and other nutrients.

Rice development stage was assessed at each sample time. From the R0 (panicle initiation; Counce et al., 2000) to R2 (50% of plants with a fully emerged flag leaf with visible collar) stages, eight or more main rice stems (with roots) were collected from each trial, stems were cut longitudinally and the internode elongation distance from the bottom node to the top node was measured. The DD10 program predicts the date of 1.25-cm internode elongation which

approximates the panicle differentiation stage (R1 stage; Hardke & Norman, 2018). As rice approached the R2 stage, the percentage of the 20 sampled plants with a fully emerged flag leaf (R2 stage) or partially emerged panicle (R3 stage) was recorded in each plot during sample collection. The actual dates of 1.25-cm internode elongation and 100% flag leaf emergence and 50% heading were extrapolated from these measurements. The measured mean internode elongation distance for the first 2 wk after internode movement was regressed against the number of days between measurements using a linear model. The mean daily internode movement was 3.8 mm d^{-1} in 2018 (n = 9, R^2 = 0.97) and 4.0 mm d^{-1} in 2019 (n = 21, R^2 = 0.80).

Rice Yield

Rice grain yield was measured by harvesting the middle five rows of each plot (3.5 m^2) using a small-plot combine. Grain moisture was determined for each plot from a subsample of grain and grain yield was standardized to a uniform moisture content of 120 g H_2O kg^{-1} for statistical analysis. The relative yield was calculated to standardize grain yield (0-100%) among trials to remove yield biases caused by potential differences of year, cultivar, environment, seeding date, management, or combinations of these factors. Relative yield was calculated for each replicate by dividing the individual plot yield of each block by the highest yielding treatment.

Statistical Analysis

Yield effect from K fertilization

Each trial was a randomized complete block design with four blocks used to collect grain yield and plant tissue-K concentration data. Grain yield data from each trial were analyzed separately to determine the effect of K fertilization on rice grain yield. The ANOVA for yield data included all of the fertilizer-K rates included in each trial although plant tissues were not

collected from some treatments. The ANOVA was performed with the GLIMMIX procedure of SAS (v9.4, SAS Inst., Cary, NC). Significant treatment differences among yield means were compared using LSMEANS ($\alpha = 0.05$).

Relationships of Y-leaf Sap-K by HKIM, Sap-K by Digestion, and Y-leaf-K Concentrations

The Y-leaf sap-K concentration determined by HKIM was compared to Y-leaf-K concentration determined by tissue digestion and analysis by ICP-AES. The K concentrations of complementary samples collected from the same plots from six to eight sample dates (Table 3.2) provided a wide range of K concentrations, plant ages, and a combination of soil- and fertilizer-K availability levels. The sap-K concentrations determined by HKIM were regressed against Y-leaf-K concentration using the REG procedure of SAS (v9.4, SAS Inst., Cary, NC) to examine the fit of linear and quadratic models assuming a normal distribution.

The relationship between sap- and leaf-K concentration was also compared for four subsets of data to examine whether the relationships were consistent among the two long-term trials (Trials A and F) and the short-term trials conducted in 2018 (Table 3.1) where data were pooled by cultivar type (hybrid or pure line). Regression was performed using the GLIMMIX procedure of SAS (v9.4, SAS Inst., Cary, NC) with a gamma distribution and a log transformation of Y-leaf-K and sap-K concentration data. The Kenward Rogers option was used for computing the denominator degrees of freedom for fixed effects. Differences among the cultivars and years were compared using ESTIMATE statements with significant differences identified at $\alpha=0.05$. The regression process for the four data subsets was repeated using the REG procedure assuming a normal distribution to numerically compare the R^2 values to the all-data relationship.

A subset of 50 sap samples from the 2018 trials was selected for additional analysis to examine the accuracy of K concentrations measured by the HKIM instrument. The sap samples were from the five trials conducted during the 2018 growing season and represented a range of sap-K concentrations by HKIM. The sap-K concentrations determined by HKIM were regressed against the sap-K concentrations determined after sap digestion and analysis by ICP-AES (Table 3.3) using the REG procedure of SAS (v9.4, SAS Inst., Cary, NC) to examine the significance of linear and quadratic models.

Y-leaf Sap-K and Y-leaf-K Concentration as Affected by K Rate and Sample Time

The K-concentration data for Y-leaf tissue determined by ICP-AES and sap-K concentration determined by HKIM from each trial were used to examine the trend in Y-leaf-K concentrations across time and determine if Y-leaf-K concentrations could differentiate among fertilizer-K rates and cultivars. Regression was performed on measurements taken between 0 and 640 GDU after the R1 stage (DD10R1) using the GLIMMIX procedure of SAS (v9.4, SAS Inst., Cary, NC) with a gamma distribution and a log transformation of Y-leaf-K and sap-K concentration data. The Kenward Rogers option was used for computing the denominator degrees of freedom for fixed effects. The DD10R1 time unit was divided by 100 (DD10RH) for SAS to produce estimable coefficients and standard errors. The Y-leaf-K and sap-K concentrations from replicate observations were regressed across DD10RH allowing for linear and quadratic DD10RH terms with coefficients depending on the cultivar (Trials B and C) when only a single fertilizer-K rate was sampled, fertilizer-K rate (Trials A and F) for the two long-term trials, or cultivar and fertilizer-K rate (Trials D and E) when both cultivar types and multiple fertilizer-K rates were sampled. A final model for each of the four datasets was derived by sequentially removing the most complex non-significant model terms (P>0.10). The Cooks D

and studentized residual (±2.5) statistics were used to identify influential and outlying data points, respectively, which were subsequently removed from the dataset and the model was refit. A pairwise analysis of fertilizer-K rates, cultivars, or times (DD10RH) was performed using the 95% confidence limits of the prediction at selected points of interest.

Correlation of Grain Yield with Sap-K Concentration

Continuous, critical HKIM sap-K and leaf-K concentrations were determined using a multiple regression model in GLIMMIX (SAS v9.4, SAS Inst., Cary, NC) for pure-line cultivar plant data collected between 0 and 640 DD10R1. Regression analysis was performed on data from four trials planted to a pure-line cultivar because the two trials seeded with a hybrid cultivar did not respond to K fertilization suggesting the hybrid may respond differently to K fertilization than the pure-line cultivar. The relative yield was regressed across the linear and quadratic terms of cumulative DD10RH and HKIM sap-K or leaf-K concentration plus the linear and quadratic interaction terms involving cumulative DD10RH and sap- or leaf-K concentration using a gamma distribution and log transformation of relative yield data. The Kenward Rogers option was used for computing the denominator degrees of freedom for fixed effects. The final model for each dataset was derived by sequentially removing the most complex non-significant model terms (P>0.10) and rerunning the model. The Cooks D and studentized residual (±2.5) statistics were used to identify influential and outlying data points, respectively, which were subsequently removed from the dataset and the model was refit.

The Y-leaf-K concentrations that produced 90 and 95% maximum yield were predicted using the final model for pure-line cultivars. The 90 and 95% of maximum predicted yield thresholds were selected to represent the plant nutrition levels of 'Deficient' when <90%, 'Probable Deficiency' or 'Low' K when 90 to 95%, and 'Sufficient' when >95% of maximum

yield. These three levels fit within the concepts outlined by Dow and Roberts (1982) and Ulrich and Hills (1973) describing plant growth or yield as affected by nutrient concentrations.

Critical K concentrations were also assessed using a second modeling approach that allocated data into overlapping time intervals for consecutive 100 DD10R groups (e.g., 0-100, 50-150, 100-200, etc) with each interval overlapping by 50 DD10R. An interval of 100 DD10R was selected since it represents about 5.5 calendar days (maximum of 17.8 DD10 d^{-1}). For each DD10R interval, relative yield was regressed across the sap-K concentrations using a quadratic model, which was simplified to a linear model when the quadratic coefficient was not significant ($P \leq 0.10$) using the REG procedure (SAS v9.4, SAS Inst., Cary, NC). The final model was used to predict the sap-K concentrations that produced 95% of the maximum predicted yield for the midpoint of each time interval which were regressed across cumulative GDUs with a model that included the linear and quadratic time terms.

Results and Discussion

Rice Yield Response to K Fertilization

Rice grain yield was significantly affected by the fertilizer-K rate at three (trials A, D, and F) of the six sites (Table 3.4). At the three K-responsive trials, rice receiving no fertilizer K produced the lowest yield, which was 66 to 81% of the maximum yield. The statistically greatest yields were produced by rice receiving ≥75 kg K ha^{-1}. Rice fertilized with 37 kg K ha^{-1} produced intermediate yields that were greater than the yields of rice receiving no fertilizer K and lower than rice fertilized with ≥75 kg K ha^{-1}. Based on the mean soil-test K in the no-K control of each site and the published critical soil-test K of 99 mg K kg^{-1} (Slaton et al., 2009), yield increases to K fertilization were expected in trials A, D, E, and F. Trials B and C had mean Mehlich-3 extractable K values >100 mg K kg^{-1} and were not expected to respond positively to K

fertilization. Trial E was expected to respond positively to K fertilization but K fertilization neither benefitted nor harmed rice grain yield. The only differences from the adjacent Trial D were that Trial E was seeded with a hybrid cultivar, Gemini 214 CL (Table 3.1), and the fertilizer-K rate treatments were slightly different (Table 3.4). The mixture of responsive and non-responsive trials is ideal for examining whether plant-K concentration determined via Y-leaf sap extraction or traditional lab analysis has utility for identifying K deficiency and predicting when grain yield increases to K fertilization will occur. The reasons why there was no benefit from K fertilization on rice grain yield in tTrial E is not clear, but Fryer et al. (2019) stated the 'false positive' was most common error in soil-test-based recommendations. Alternatively, the majority of published soil-test K correlation and calibration trials have been performed with pure-line cultivars and perhaps hybrid cultivars respond differently to native soil fertility and fertilization. Yang et al. (2003) showed that rice genotypes may contain different leaf-K concentrations and respond differently to K fertilization.

Sap-K by HKIM Compared to Traditional Leaf-K Testing

The relationship between Y-leaf-K concentration determined by ICP-AES and sap-K concentration determined by HKIM for all data (n=371) was positive and linear but the coefficient of determination (r^2) was only 0.39 (Fig. 3.1). Although the two K concentration measurements are significantly related, the relationship across all sample times, fertilizer-K rates and trials is relatively weak. The observations were parsed into four categories that isolated data from the long-term trials in each year and the short-term trials by cultivar in 2018 to examine whether the relationships were different among datasets. The relationship for each of the four datasets was linear and the r^2 values ranged from 0.12 to 0.52 suggesting that the relationship between leaf-K and sap-K concentrations was not consistent among individual trials or trials

having similar agronomic factors (Table 3.5). The comparison of regression coefficients among the four datasets using GLIMMIX supports the conclusion that the relationship was sometimes different. The datasets that shared similar intercepts and slopes provide little insight regarding what may have caused the differences.

A subset of 50 samples collected in 2018 that had sufficient volume for further laboratory analysis was used to examine the variability between the sap- and leaf-K concentration measurements (Table 3.3). The relationship between Y-leaf-K concentration determined by ICP-AES and sap-K concentration determined by HKIM had an r^2 of 0.54 and produced numerically similar coefficients as the analysis that used all 371 observations (Fig. 3.2A). The r^2 value improved to 0.65 when the sap-K concentration determined by HKIM was replaced with the digested sap-K concentration suggesting there may be potential issues with the handheld instrument used to measure sap-K concentration (Fig. 3.2B). The relationship between sap-K concentrations measured with the HKIM versus the digested sap and measured via ICP-AES was linear and had an r^2 of 0.87 (Fig. 3.2C). The intercept and slope coefficients indicate the handheld HKIM tends to underestimate the sap-K concentration, especially when sap-K concentrations are relatively high (Fig. 3.2C).

Tabor and Lawson (2007) and Gangaiah et al. (2015) both showed that the sap-K concentration (diluted or undiluted) measured on the Cardy meter and leaf-K concentration by ICP-AES were linearly related but had considerable variance across the range of concentrations. Taber and Lawson (2007) and Rosen et al. (1996) reported quadratic relationships between the undiluted sap-K concentrations of tomato and potato, respectively, measured by the Cardy meter and sap-K concentration after digestion and determination by ICP-AES. Tabor and Lawson (2007) suggested the concentrations were linear and strong ($r^2 = 0.94$) up to 3000 mg K L^{-1}, but

the relationship weakened at higher (>3000 mg K L^{-1}) sap-K concentrations with greater variance (r^2 = 0.54). Research by Rosen et al. (1996) and Taber and Lawson (2007) showed that diluting the sap with deionized water before measurement on the Cardy meter resulted in linear relationships with a higher r^2 and slopes near 1.0 across the range of sap-K concentrations as compared to undiluted sap. The literature combined with our data for rice suggests that sap-K concentration measured by a handheld device qualitatively approximates leaf-K concentration and is more accurate across the range of concentrations when diluted before measurement. We assume that the sampling protocols used to select and divide leaf samples for each analytical process is not biased or flawed. The literature does not address the precision of the Cardy meter or the HKIM, but our experience is that the HKIM instrument provides consistent readings on subsamples from the same sap sample suggesting that the process of extracting sap from plant leaves may be variable and require additional research to enhance uniformity. We have observed differences in sap-K concentrations when the rice or soybean leaf sap was extracted using a hand-operated garlic press or a hydraulic press compared to the manufactured hitch press used for this study (unpublished data).

Sap-K Concentrations as Affected by Cultivar and Fertilizer-K rate Across Time

The pure-line and hybrid cultivars planted in adjacent areas in the same field in trials B and C and trials D and E were compared to evaluate the effect of cultivar on rice Y-leaf sap-K concentrations across time (Fig. 3.3 & Table 3.6). In trials B and C, the sap-K concentration for rice fertilized with 0 kg K ha^{-1} was greater in the hybrid cultivar than the pure-line cultivar for the first 100 DD10R1 (Fig 3.3A). The sap-K concentration of both cultivars decreased linearly across time but the change across time was numerically greater for the hybrid (Δ1830 mg K L^{-1}) than the pure-line (Δ768 mg K L^{-1}). Trials D and E showed no difference between cultivars when

the same (0 kg K ha^{-1}) or similar (37 vs 47 and 140 vs 150 kg K ha^{-1}) fertilizer-K rates were compared at the same time points (Fig. 3.3B & 3.3C). We could find no published information comparing the effect of cultivar on sap-K concentrations, but Yang et al. (2003) showed that rice genotypes may contain different leaf-K concentrations and respond differently to K fertilization. Thus, differences in sap-K concentration among cultivars seem likely.

Sap-K concentrations showed no consistent trend across time for the six trials (Fig. 3.3 & 3.4). The sap-K concentrations of rice that received no fertilizer-K in trials B and C declined linearly across time. However, sap-K concentration was constant across time in trials D and E with the predicted sap-K concentrations fluctuating by 122-457 mg K L^{-1} when no fertilizer K was applied. Rice receiving no fertilizer-K in trials A and F showed positive or negative, respectively, quadratic responses across time (Fig. 3.4) with the predicted sap-K concentration changing by 1762 mg K L^{-1} for Trial A and 1012 mg K L^{-1} for Trial F (Table 3.6). There was no consistency in trends across time when examined by rice grain yield response to fertilizer-K rate (Table 3.3; Fig. 3.3 & 3.4).

Rice that received the intermediate and high fertilizer-K rates also failed to show consistent sap-K concentration trends across time among the trials (Fig. 3.3 and 3.4). The Y-leaf sap-K concentration differences among fertilizer-K rates were distinguishable only for the first 100 DD10R1 and were different only between rice that received the greatest fertilizer-K rate and no fertilizer K. These trends suggest that the sap-K concentration measured by the HKIM may have limited utility as a rapid in-field method for diagnosing K deficiency of rice due to the inconsistencies in sap-K trends across time. The inconstancies may be attributed to the variability in concentrations due to sap-K being a more sensitive measure of K nutrition (Joris, Souza, Montezano, Vargas, & Cantarella, 2014).

The Y-leaf-K concentration appears to be a better measure of rice K nutritional status than sap-K concentration but Y-leaf-K concentration may only be diagnostic before the R2 stage since leaf-K concentrations were also hard to distinguish among fertilizer-K rates after the R2 stage. Dunn et al. (2004) is the only published research we could find reporting results of sap-K concentrations for rice and they concluded that sap was not a feasible method of monitoring rice K nutrition because it was almost impossible to extract from rice leaves. We showed that sufficient sap could be extracted from rice leaves with the proper equipment but the sap-K concentrations measured by the HKIM were too variable to be useful. Despite our conclusions about the utility of sap-K concentration for rice, fresh sap is extracted and analyzed for K concentrations with handheld meters and used to monitor the K nutrition of many dicots including eggplant, pepper, potato, tomato, and watermelon (Hochmuth, 1994). Additional research may be required to perfect the methodology for rice and other monocots.

Leaf-K Concentrations as Affected by Cultivar and Fertilizer-K rate Across Time

The Y-leaf-K concentration trend across time (Fig. 3.5 & 3.6) was more consistent among trials than what was observed for sap-K concentration (Fig. 3.3 & 3.4). Except for trials B and C (Fig. 3.5A), the leaf-K concentrations can be generalized as constant across time for rice receiving no fertilizer-K (0 kg K ha^{-1}) or intermediate fertilizer-K rates (37-47 kg K ha^{-1}) and to decrease linearly or quadratically across time for rice that received relatively high fertilizer-K rates (75-150 kg K ha^{-1}; Fig. 3.5 & 3.6). In trials B and C (Fig. 3.5A), the leaf-K concentrations of rice receiving no fertilizer-K declined linearly (Trial C) or quadratically (Trial B) across time presumably because the available soil-K in this field was greater than in the other fields (Table 3.1), which is why samples were collected only from the no fertilizer-K control in these two trials.

Rice in trials A (Fig. 3.6A), D (Fig. 3.5B), E (Fig. 3.5C) and F (Fig. 3.6B) showed significant differences in leaf-K concentrations between treatments fertilized with 0 and 140 or 150 kg K ha^{-1} until 360 DD10R1 for trials D and E and 480 DD10R1 for trials A and F. In contrast, the sap-K concentrations showed no differences between the lowest and highest fertilizer-K rates by 140 DD10R1 for trials B (Fig. 3.3B) and C (Fig. 3.3C) or 180 to 220 DD10R1 (Fig. 3.4) for trials A and F. Xue et al. (2016) reported that rice leaf-K concentrations peaked during the tillering and jointing stages and decreased through reproductive growth with the differences among fertilizer-K rates diminishing across time. Our results suggest that leaf-K concentration is better able to distinguish differences in plant-K nutrition response later into reproductive growth than sap-K concentration. Neither leaf- nor sap-K concentrations were able to consistently differentiate among fertilizer-K rates by the R3 stage, but leaf-K concentration differentiated among the lowest (0 kg K ha^{-1}) and highest (140-150 kg K ha^{-1}) fertilizer-K rates until the R2 stage, which was about one week longer than sap-K concentration. Doberman and Fairhurst (2000) and Rama Rao and Sekhon (1988) both reported critical leaf-K concentrations for rice during reproductive growth but only Rama Rao and Sekon (1988) provided research evidence to support their suggested critical concentrations. Dunn et al. (2004) reported that the basal stem sap-K concentration measured on the Cardy meter or the K-concentration of the Y-leaf or basal stem as determined by atomic absorption spectroscopy at the R1 stage was near equally correlated (R^2 = 0.24 to 0.31) with the rice yield. However, the correlation decreased substantially for both basal stem-K concentrations at the R2 development stage, but not for the Y-leaf-K concentration.

Grain Yield Correlation with Sap-K and Leaf-K Concentration and Time

Relative yield as affected by sap-K (*n*=247) or leaf-K (*n*=241) concentrations and DD10R1 focused on pure-line cultivars (trials A, B, D, & F) because the results suggested that hybrid cultivars may respond differently to K fertilization than the pure-line cultivar. The multiple regression models for sap-K (Fig. 3.7A) and leaf-K concentration (Fig. 3.7B) were both significant (Table 3.8), but the model for Y-leaf-K concentration was a better fit as evidenced by having a generalized Chi-squared value closer to 1 (Y-leaf-K = 0.92; Sap-K = 2.37). The sap-K model suggested a sap-K concentration of about 3900 mg K L^{-1} was critical at R1 and the critical-K concentration increased to 3500 mg K L^{-1} at the R2 stage (Fig. 3.7A). The Y-leaf-K concentration model showed that the critical Y-leaf-K concentration at the R1 stage was 13.89 g K kg^{-1}, gradually increased to a peak of about 17.49 g K kg^{-1} at 358 DD10R1, and then declined to about 15.23 g kg^{-1} by the R3 stage (Fig. 3.7B).

The interval-specific regression shows that the coefficient of determination was numerically greater for leaf-K predictions as compared to sap-K predictions for each of the 12 DD10R1 intervals (Table 3.9). Sap-K concentration explained 29 to 41% of the variability in relative rice yield between 0 and 300 DD10R1 compared to 60 to 83% of the variability explained by Y-leaf-K concentration. Unlike the multiple regression model, the individual time interval predictions suggested the critical sap-K concentration was 4230 mg K L^{-1} at the R1 stage and decreased to 3309 mg K L^{-1} between 300 and 400 DD10R1, which approximates the R2 stage, and 2746 mg K L^{-1} between 500 and 600 DD10R1, the interval that includes the R3 stage. Predictions with sap-K concentration made beyond 300 DD10R1 explained only 0.06 to 0.26% of the variability in relative yield.

The only other published research investigating the use of sap to monitor rice K nutritional status by Dunn et al. (2004) also showed that the coefficient of determination between rice yield and basal stem sap-K concentration was greatest at R1 (0.30) and decreased as rice progressed to the R2 (0.12) stage. Dunn et al. (2004) also showed the Y-leaf-K concentration was superior to sap-K at the R2 stage. Despite recommendations for the use of sap-K concentration to monitor plant K nutrition (Hochmuth, 1994), the literature contains little information describing the relationship between crop yield and sap-K concentration. Mohr and Tomasiewicz (2012) show that the relationship between potato yield and the sap-K concentration extracted from petioles 82 to 85 d after planting was relatively weak ($r^2 = 0.24$). The inability of the handheld devices, like the HKIM and Cardy meter, to accurately measure undiluted sap-K concentration shown by us (Fig. 3.1 & 3.2), Nagarajah (1999), Rosen et al. (1996), and Tabor and Lawson (2007) may be an important factor contributing to the poor relationship between relative rice yield and sap-K concentration.

Conclusions

Our research examined the relationship between rice Y-leaf tissue- and sap-K concentrations during reproductive growth and their utility for monitoring plant K sufficiency status from the R1 through R3 development stages of rice grown in the direct-seed, delayed-flood production system. The research with rice Y-leaf sap K concentration is novel in that the literature contains only one published, albeit brief, account of research examining the extraction of sap from rice tissues and its correlation to relative rice yield. Although the extraction of sap from the rice Y-leaf is difficult, it can be done with the proper equipment. The sap-K concentration as measured by the handheld HKIM was weakly correlated with leaf-K concentration and weakly correlated with relative rice yield between the R1 and R2 development

stages. The Y-leaf tissue K concentration as determined by standard digestion and analysis in the laboratory proved to be a more accurate indicator of the relative yield of a pure-line rice cultivar and to differentiate among fertilizer-K rates within a trial than sap-K concentration. While the sap-K concentration can be obtained more rapidly and determined in the field within minutes of sap extraction on the handheld HKIM, the method of extraction may need to be improved to be less variable and the sap may need to be diluted with deionized water to reduce the ionic strength of the solution before reading on the HKIM. Improving the consistency of sap-K extraction may improve the relationship with leaf-K concentration and its accuracy as an index of the K nutritional status of rice.

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Tables and Figures

Table 3.1. Selected site, treatment, and soil characteristics of six trials located at the Pine Tree Research Station (PTRS) near Colt, AR during 2018 and 2019.

							Mehlich-3 nutrients[e]				
Trial[a]	Year	Cultivar[b]	Soil Series	Fertilizer-K rate	Soil pH[c]	Soil O.M.[d]	P	K	Ca	Mg	Zn
				kg K ha^{-1}		g kg^{-1}	----------		mg kg^{-1}	----------	
A	2018	Diamond	Calhoun	0	8.1	25.7	42	32	3361	412	9.7
				37	8.1	-	39	51	3318	428	9.1
				150	8.0	-	35	84	2875	397	9.4
B	2018	Diamond	Calloway	0	6.4	25.6	29	111	1274	214	1.8
C	2018	Gemini	Calloway	0	6.4	22.8	31	103	1219	197	1.6
D	2018	Diamond	Calloway	0	7.6	23.1	14	66	2238	324	1.4
E	2018	Gemini	Calloway	0	7.7	22.7	13	68	1919	316	1.4
F	2019	Diamond	Calhoun	0	8.1	27.3	37	50	3335	435	7.0
				37	8.3	-	31	47	3305	421	6.2
				75	8.0	-	30	63	3113	430	6.9
				150	8.1	-	30	76	3171	431	6.8

[a]Trials A & F are long-term trials, Trials B & D are short-term (1 year) trials, and Trials C & E are short-term trials.
[b]Diamond is a pure-line rice cultivar and Gemini 214 Clearfield is a hybrid rice cultivar.
[c]Soil pH measured in a 1:2 (v:v) soil:water mixture (Sikora & Kissel 2014)
[d]O.M.= organic matter, Schulte & Hopkins (1996)
[e]Zhang, Hardy, Mylavarapu, & Wang (2014)

Table 3.2. Selected dates of agronomic importance for six trials conducted during 2018 and 2019 located at the Pine Tree Research Station near Colt, AR.

	Management and growth stage[b] dates					Tissue sample collection dates							
Trial[a]	Emerged	Flooded	R1	R2	R3	1	2	3	4	5	6	7	8
A	5 May	1 June	20 June	11 July	21 July	20 June	27 June	3 July	10 July	17 July	24 July	1 Aug.	9 Aug.
B	4 May	1 June	20 June	11 July	22 July	20 June	28 June	3 July	10 July	17 July	25 July	-	-
C	4 May	1 June	19 June	11 July	21 July	19 June	28 June	3 July	10 July	17 July	25 July	-	-
D	1 May	31 May	19 June	11 July	23 July	19 June	27 June	3 July	10 July	17 July	25 July	-	-
E	2 May	31 May	13 June	9 July	20 July	19 June	27 June	3 July	10 July	17 July	25 July	2 Aug.	-
F	5 May	4 June	3 July	20 July	29 July	25 June	2 July	9 July	17 July	23 July	30 July	7 Aug.	13 Aug.

[a]Trials A, B, D, & F were seeded with a pure-line (Diamond) cultivar and Trials C & E hybrid were seeded with a hybrid cultivar (Gemini 214 CL).

[b]The R1 stage is when internode spacing reaches 12.7 mm, the R2 stage is defined as 50% of the flag leaf collars are visible, and the R3 stage is when 50% of the plants have a panicle exerted above the flag leaf collar (Counce et al., 2000).

Table 3.3. Selected information showing the source of 50 Y-leaf sap and tissue samples comparing K concentrations of undiluted sap analyzed with the Horiba K ion meter (HKIM), digested sap (Digested) and digested Y-leaf tissue from five trials conducted in 2018.

			Sap-K concentration						Sap-K concentration		
Sample No	Trial ID	Sample date	HKIM[a]	Digested[b]	Y-leaf-K[b]	Sample No	Trial ID	Sample date	HKIM[a]	Digested[b]	Y-leaf-K[b]
			mg K L^{-1}		g K kg^{-1}				mg K L^{-1}		g K kg^{-1}
1	D	19 June	5000	5756	18.95	26	D	27 June	2800	2890	12.08
2	D	19 June	3400	3558	10.81	27	D	27 June	2900	2941	10.85
3	D	19 June	3300	3295	9.49	28	D	27 June	3900	4687	15.13
4	D	19 June	4600	5466	15.67	29	D	27 June	2900	3139	11.64
5	D	19 June	3200	3698	13.86	30	D	27 June	4300	5128	16.84
6	D	19 June	4600	5597	18.00	31	D	27 June	2700	3092	10.08
7	B	20 June	3200	4176	20.93	32	C	28 June	4000	5830	23.70
8	B	20 June	3600	4837	19.62	33	C	28 June	3900	6002	21.88
9	B	20 June	3800	5369	21.76	34	C	28 June	3200	4309	22.51
10	B	20 June	3600	5002	20.53	35	C	28 June	3700	5531	22.42
11	A	20 June	4600	5395	25.45	36	C	28 June	4400	6579	22.95
12	A	20 June	4000	4709	30.46	37	A	27 June	3900	4696	14.95
13	A	20 June	2600	2796	11.20	38	A	27 June	4900	6618	22.51
14	A	20 June	2600	2914	12.16	39	A	27 June	4100	4925	14.39
15	A	20 June	2700	2770	12.78	40	A	27 June	4000	5129	14.39
16	A	20 June	4900	6435	28.68	41	A	27 June	4800	6316	23.04
17	A	20 June	2200	2273	9.14	42	A	27 June	4100	5183	17.12
18	A	20 June	4500	5662	23.70	43	E	25 July	2000	2438	9.94
19	A	20 June	2300	2369	14.03	44	E	25 July	2100	2771	10.18
20	D	27 June	3600	3875	11.42	45	E	25 July	2600	3480	10.94
21	D	27 June	3600	3933	17.77	46	E	25 July	2000	2561	9.22
22	D	27 June	2900	2947	13.24	47	E	25 July	2500	3412	9.37
23	D	27 June	2600	2644	11.98	48	E	25 July	2000	2581	11.33
24	D	27 June	3100	3180	10.48	49	E	25 July	2500	3272	9.24
25	D	27 June	3800	4332	18.04	50	E	25 July	3100	3938	10.27

[a]Undiluted sap samples were analyzed on the Horiba Laquatwin K meter (Horiba Instruments Inc., Kyoto, Japan

[b]Sap and Y-leaf tissue samples were digested using HNO_3 and H_2O_2 and analyzed with inductively coupled plasma atomic emission spectrophotometry.

Table 3.4. Grain yield for pure-line and hybrid rice cultivars planted in six field trials at the Pine Tree Research Station (PTRS) near Colt, AR conducted in 2018 and 2019.

		Fertilizer-K rate (kg K ha^{-1})					
Trial[a]	Cultivar[b]	0	37/47[c]	75/93	112	150/140	*P*-value
		---------- Grain yield (kg ha^{-1}) ----------					
A	Diamond	8,366c[d]	10,076b	10,588ab	11,062a	11,153a	0.0002
B	Diamond	10,522	10,564	10,027	10,040	10,683	0.2048
C	Gemini	12,168	12,305	12,396	-	12,572	0.2326
D	Diamond	8,760c	9,886b	10,214ab	10,323ab	10,821a	0.0002
E	Gemini	11,715	11,311	11,886	-	11,322	0.3292
F	Diamond	6,228c	7,864b	8,991a	9,440a	9,457a	<0.0001

[a]Soil-test information for each trial is shown in Table 3.1.

[b]Diamond is a pure-line cultivar and Gemini 214 CL is a hybrid cultivar.

[c]When two fertilizer-K rates are listed in the same column the first listed rate was used for trials where Diamond was the cultivar and the second listed rate was used for trials where Gemini 214 CL was the cultivar.

[d]Within a row, yield means followed by different lowercase letters are statistically different at the 0.05 level.

Table 3.5. Linear regression coefficients and standard errors describing the relationship between leaf-K and sap-K concentrations from four datasets from research located at the Pine Tree Research Station (PTRS) near Colt, AR in 2018 and 2019.

		Regression coefficients[b]				
Dataset[a]	Observations	Intercept	SE	Linear	SE	r^{2c}
	n					
2018 Hybrid	108	7.52a[d]	0.066	0.034a	0.0037	0.52
2018 Pure-line-A	96	7.82b	0.070	0.016	0.0046	0.12
2018 Pure-line-B	72	7.73b	0.066	0.026	0.0042	0.26
2019 Pure-line	96	7.45a	0.060	0.033a	0.0046	0.44

[a]The data pooled for the four datasets included: Trials C and E for '2018 Hybrid' seeded with Gemini 214 CL; Trials B and D for '2018 Pure-line A' representing two single-year trials seeded with Diamond; Trial A representing data collected from the long-term in 2018 for '2018 Pure-line B'; and Trial F representing data collected from the long-term trial in 2019 for '2019 Pure-line'.

[b]Coefficients derived from linear regression in PROC GLIMMIX using a gamma distribution and log transformation of data. Approximate (due to coefficient rounding) predicted values can be calculated using the following equation: $e^{Y} = bx + a$, where Y = sap-K concentration (mg K L^{-1}); x = leaf-K concentration (mg K kg^{-1}); b = linear slope coefficient, a = intercept; and e = natural exponential function (approximately 2.718281828...).

[c]r^2 from PROC REG analysis.

[d]Coefficients are significantly different at $\alpha = 0.05$.

Table 3.6. Regression coefficients and standard errors describing rice sap-K concentration as determined by a handheld Horiba K^+ ion meter (HKIM) across time (DD10R1) as affected by cultivar, fertilizer-K rate or both for six field trials conducted at the Pine Tree Research Station (PTRS) near Colt, AR during 2018 and 2019.

		Regression coefficients (transformed)[a]			Coefficients (non-transformed)			Predictions			
Trial	Fertilizer-K rate	Intercept	Linear	Quadratic	Intercept	Linear	Quadratic	Max	Time	Min	Time
	kg K ha^{-1}										
A	0	7.84	-0.010	0.0147	2563	-0.90	0.0056	4289	640	2527	80
	37	8.00	0.062[b]	-0.0055[b]	2973	1.97	-0.0017	3540	576	2973	0
	150	8.55	-0.236	0.0305	5066	-9.42	0.0121	5066	0	3233	389
	SE	0.080	0.0671	0.01101	--	--	--	--	--	--	--
B[c]	0	8.18	-0.038	NS[d]	3565	-1.20	NS	3565	0	2797	640
C	0	8.36	-0.087	NS	4188	-2.86	NS	4188	0	2358	640
	SE	0.042	0.0116	--	--	--	--	--	--	--	--
D	0	8.05	0.040	0.0136	3124	-2.42	0.0040	3215[e]	640	2758[e]	302
	37	8.08	0.034	0.0136	3234	-2.66	0.0041	3234[e]	0	2799[e]	327
	150	8.41	-0.018[b]	0.0136	4446	-5.36	0.0053	4446	0	3099	503
	SE	0.046	0.0282	0.00395	--	--	--	--	--	--	--
E	0	8.05	-0.082	-0.0100	3132	1.21	-0.0030	3254[e]	202	3132[e]	0
	47	8.08	-0.089	-0.0100	3246	0.99	-0.0030	3328[e]	166	3246[e]	0
	140	8.41	-0.141	-0.0100	4505	-1.34	-0.0025	4505	0	2621	640
	SE	0.046	0.0263	0.00445	--	--	--	--	--	--	--
F	0	7.39	0.266	-0.0371	1575	5.69	-0.0080	2587	356	1575	0
	37	7.71	0.150	-0.0238	2214	3.78	-0.0060	2810	316	2180	640
	75	7.84	0.076	-0.0117	2525	2.06	-0.0032	2861[e]	326	2525[e]	0
	150	8.00	0.002[b]	-0.0024[b]	2981	0.03	-0.0007	2981[e]	0	2731[e]	640
	SE	0.049	0.0410	0.00696	--	--	--	--	--	--	--

[a]Coefficients derived by first dividing the DD10R1 units by 100 and regression in PROC GLIMMIX using a gamma distribution and log transformation of data. Predicted values can be calculated using the following equation: $e^Y = ax^2 + bx + c$, where Y = sap-K concentration (mg K L^{-1}); x = growing degree units after R1 stage; a = quadratic coefficient b = linear coefficient, c = intercept; and e = natural exponential function (approximately 2.718281828...).

[b]Coefficients are not significantly different from zero at $\alpha = 0.05$.

[c]Adjacent trials were analyzed together to compare fertilizer-K rates or cultivars (B and C) and fertilizer-K rates (D and E)

[d]NS, the Quadratic coefficient was not significant in the final model at $P>0.10$.

[e]Leaf-K maximum (Max) and minimum (Min.) values that are not significantly different from each other at the 0.05.

Table 3.7. Regression coefficients and standard errors for predicting Y-leaf-K concentration during reproductive growth as affected by fertilizer-K rate and time (DD10R1) for 6 trials conducted at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR and in 2018 and 2019.

	Fertilizer-K	Regression coefficients (transformed)[a]			Coefficients (non-transformed)			Predictions			
Trial[a]	rate	Intercept[b]	Linear	Quadratic	Intercept	Linear	Quadratic	Max	Time	Min	Time
	kg K ha^{-1}										
A	0	2.20	0.203	-0.0267	8.88	0.0230	-0.000031	13.22	377	9.03	0
	37	2.55	0.152	-0.0276	12.81	0.0209	-0.000038	15.71	277	10.94	640
	150	3.28	-0.116	0.0012[c]	26.45	-0.0290	0.000013	26.52	0	13.25	640
	SE	0.035	0.0266	0.00432	--	--	--	--	--	--	--
B	0	3.05	0.016[c]	-0.0146	21.18	0.0000	-0.000021	21.18	0	11.99	640
C	0	3.23	-0.104	-0.0004[c]	25.22	-0.0255	0.000010	25.22	0	12.05	640
	SE	0.022	0.0165	0.00264	--	--	--	--	--	--	--
D	0	2.09	0.253	-0.0323	7.85	0.0270	-0.000035	13.03	384	8.08	0
	37	2.32	0.219	-0.0325	10.03	0.0280	-0.000041	14.82	342	10.16	0
	150	2.79	0.089	-0.0218	16.32	0.0130	-0.000032	17.33	201	11.70	640
	SE	0.038	0.0226	0.00343	--	--	--	--	--	--	--
E	0	2.48	0.095	-0.0137	11.85	0.0120	-0.000018	13.85[d]	334	11.89[d]	0
	47	2.71	0.061	-0.0139	14.99	0.0088	-0.000020	15.97[d]	221	13.45[d]	640
	140	3.17	-0.069	-0.0032[c]	23.89	-0.0170	0.000001	23.86	0	13.46	640
	SE	0.052	0.0319	0.00457	--	--	--	--	--	--	--
F	0	1.89	0.311	-0.0509	6.54	0.0263	-0.000043	10.58	307	6.02	640
	37	2.36	0.216	-0.0444	10.66	0.0230	-0.000047	13.50	247	6.84	640
	75	2.62	0.129	-0.0345	14.02	0.0140	-0.000039	15.29	181	7.65	640
	150	2.99	-0.009[c]	-0.0181	20.11	-0.0076	-0.000016	19.84	0	8.93	640
	SE	0.049	0.0336	0.00576	--	--	--	--	--	--	--

[a]Trial, year (2018 or 2019), The two long-term pure-line trials include A (2018) and F (2019). The remaining 4 trials were conducted at two sites with a pure-line (B & D) and hybrid (C & E) cultivar planted in adjacent areas (B & C, D & E) were analyzed together to comparison cultivar type (hybrid vs pure-line).

[b]Y-leaf-K concentration (g K kg^{-1}) = intercept + [linear × (DD10R1 1/100)] + [quadratic × ($DD10R1^2$)]. Regression was performed on DD10R1 units divided by 100 and data were transformed using a gamma distribution. The Y-leaf-K concentration calculated from the regression coefficients must be back-transformed using the exponential function e^x (g K kg^{-1}).

[c]Coefficients are not significantly different from zero (*Pr*>0.05).

[d]Leaf-K maximum (Max) and minimum (Min.) values that are not significantly different from each other at the 0.05.

Table 3.8. Regression coefficients for relative yield as affected by time (DD10R1) and Y-leaf-K concentrations (YLKC) for both sap-K and leaf-K. All trials were seeded with a pure-line cultivar and located at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR and the Rice Research and Extension Center (RREC) near Stuttgart, AR in 2018 and 2019.

	Coefficients[a]						
Model term	Intercept	DD10R1	YLKC	DD10R1× YLKC	$DD10R1^2$	$YLKC^2$	$DD10R1^2$ ×$YLKC^2$
Sap-K	4.24[b]	0.0439	0.00008	-0.00001	NS[c]	NS	NS
SE	0.044	0.01478	0.000014	0.000005	--	--	--
Leaf-K	3.65[d]	-0.1741	0.1028	0.00802	0.0200	-0.0027	-0.0001
SE	0.058	0.02940	0.00774	0.002142	0.00308	0.00024	0.00002

[a]Regression was performed on DD10R1 units divided by 100 and data were transformed using a gamma distribution. The Y-leaf-K concentration calculated from the regression coefficients must be back-transformed using the exponential function e^x (% relative yield). a = intercept, b = DD10R1, c = YLKC, d = DD10R1×YLKC, e = $DD10R1^2$, f = $YLKC^2$, g = $DD10R1^2$×$YLKC^2$.
[b]Multiple regression equation for Leaf-K: % Relative yield = a + (b×DD10RH) + (c×Leaf-K) + (d×DD10RH×Leaf-K) + (e×$DD10RH^2$) + (f ×Leaf-K^2) + (g×$DD10RH^2$× Leaf-K^2).
[c]NS, coefficient was not significant (*Pr*>0.10).
[d]Multiple regression equation for Sap-K: % Relative yield = a + (b×DD10RH) + (c×Sap-K) + (d×DD10RH×Sap-K). The relative yield calculated from the regression coefficients must be back-transformed using the exponential function e^x (% relative yield).

Table 3.9. Regression coefficients predicting relative rice yield as affected by sap-K or leaf-K concentration for 12 consecutive overlapping intervals of accumulated growing degree units after the R1 growth stage (DD10R1) using data from four trials (Trial A, B, D, and F) seeded with a pure-line cultivar in 2018 and 2019.

		Sap-K concentration						Leaf-K concentration					
		Regression coefficients [a]				Predictions [b]		Regression coefficients [a]				Predictions [b]	
DD10R1	*n*	r^2	Intercept	Linear	Quadratic	RY	Sap-K	r^2	Intercept	Linear	Quadratic	RY	Leaf-K
						%	mg L^{-1}						
0-100	28	0.36	64	0.0075	NS[c]	95.0	4133	0.60	46.61	4.63	-0.1008	94.8	15.94
50-150	44	0.29	29	0.0315	-0.0000038	90.2	3109	0.83	3.99	9.80	-0.2519	94.3	14.99
100-200	44	0.29	29	0.0315	-0.0000038	90.2	3109	0.83	3.99	9.80	-0.2519	94.3	14.99
150-250	44	0.41	-35	0.0728	-0.000010	92.0	2899	0.72	7.16	8.65	-0.2033	94.2	16.33
200-300	44	0.41	-35	0.0728	-0.000010	92.0	2899	0.72	7.16	8.65	-0.2033	94.2	16.33
250-350	31	0.11	56	0.0106	NS	95.0	3679	0.83	-6.40	8.60	-0.1560	106.4	21.51
300-400	43	0.11	58	0.0097	NS	95.0	3814	0.77	23.97	4.13	NS	95.0	17.20
350-450	28	0.26	-223	0.1974	-0.000031	88.6	2890	0.55	-52.61	18.07	-0.5554	89.7	13.37
400-500	43	0.20	-279	0.2505	-0.000042	88.4	2601	0.56	27.38	4.41	NS	99.2	16.29
450-550	27	0.06	-97	0.1330	-0.000023	88.1	2333	0.62	-333.79	52.12	-1.5720	93.3	14.81
500-600	32	0.17	-33	0.0758	-0.000011	90.6	2649	0.23	57.51	2.77	NS	92.3	12.56
550-640	44	0.12	-8	0.0593	-0.000009	88.2	2889	0.24	55.60	2.95	NS	93.2	12.75

[a]Linear ($y = a + bx$) and quadratic ($y = a + bx + cx^2$) models where y = relative yield (%), x = Y-leaf-K concentration expressed as (g K kg^{-1}), a = intercept coefficient, b = linear slope coefficient, and c = quadratic slope coefficient.

[b]95% of maximum yield calculated by multiplying the predicted maximum yield by 0.95.

[c]Linear regression was used when quadratic was not significant (p=0.11).

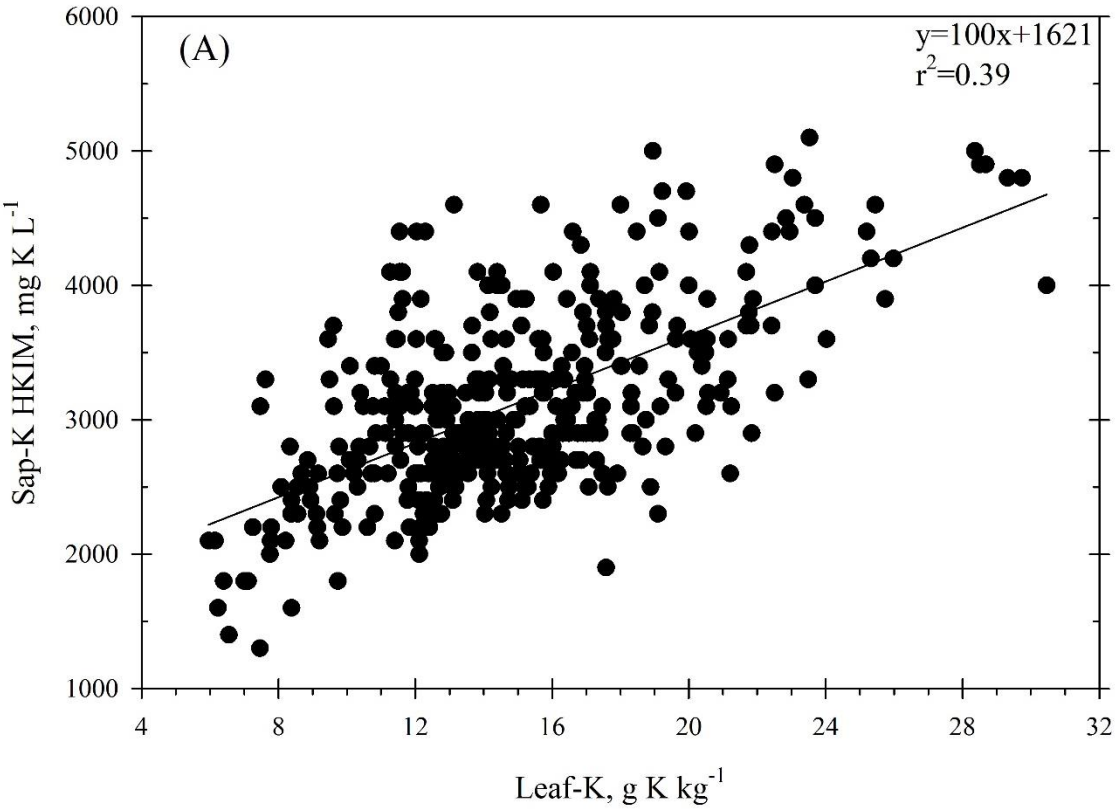

Fig. 3.1. Comparison of leaf-K and sap-K HKIM data from all trials (Trials A, B, C, D, E, & F; n=371) in 2018 and 2019 located at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR.

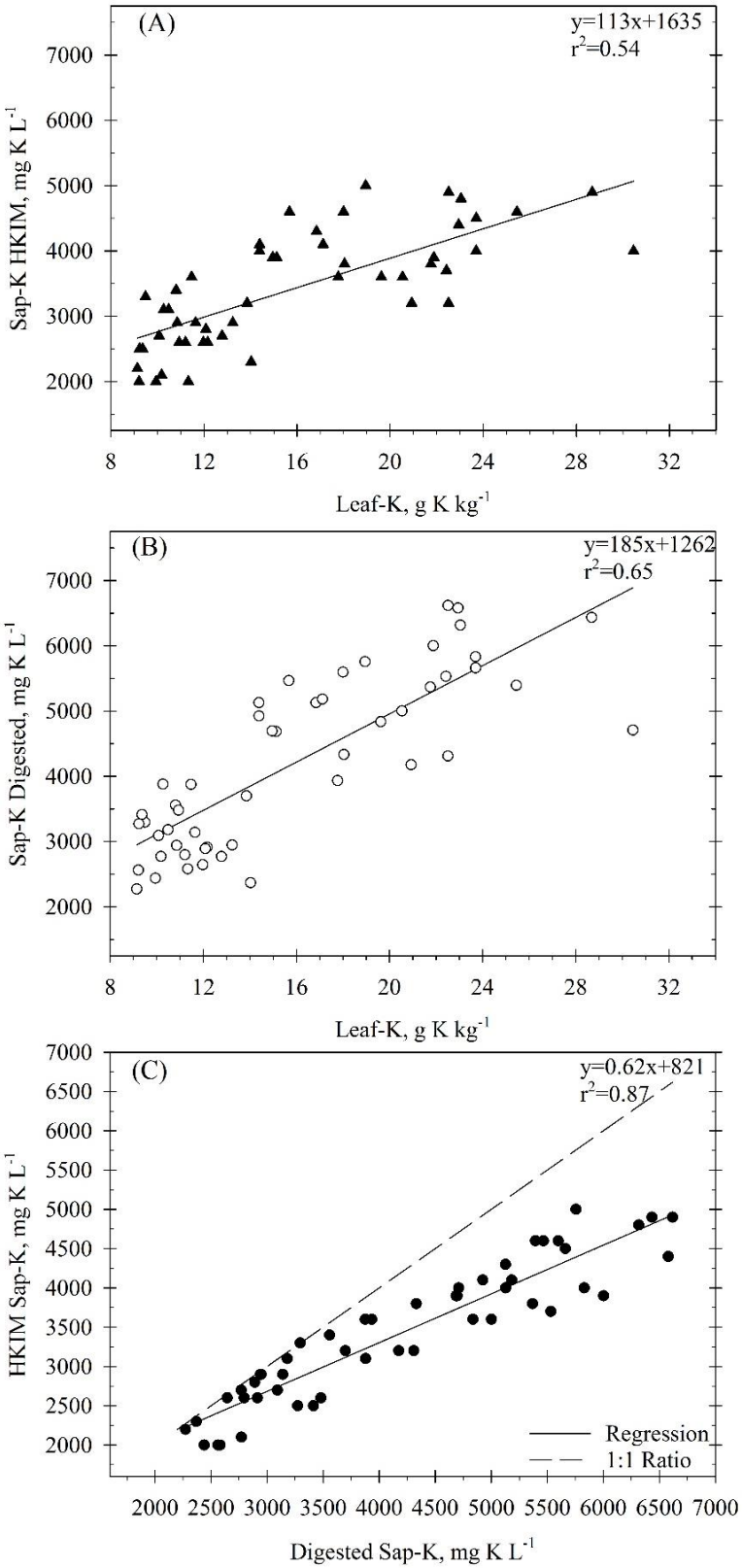

Fig. 3.2. Regression comparison of 50 samples for the K concentrations in undiluted fresh sap, analyzed with the Horiba K ion meter (HKIM), digested sap (Digested) and digested Y-leaf-K (Leaf-K).

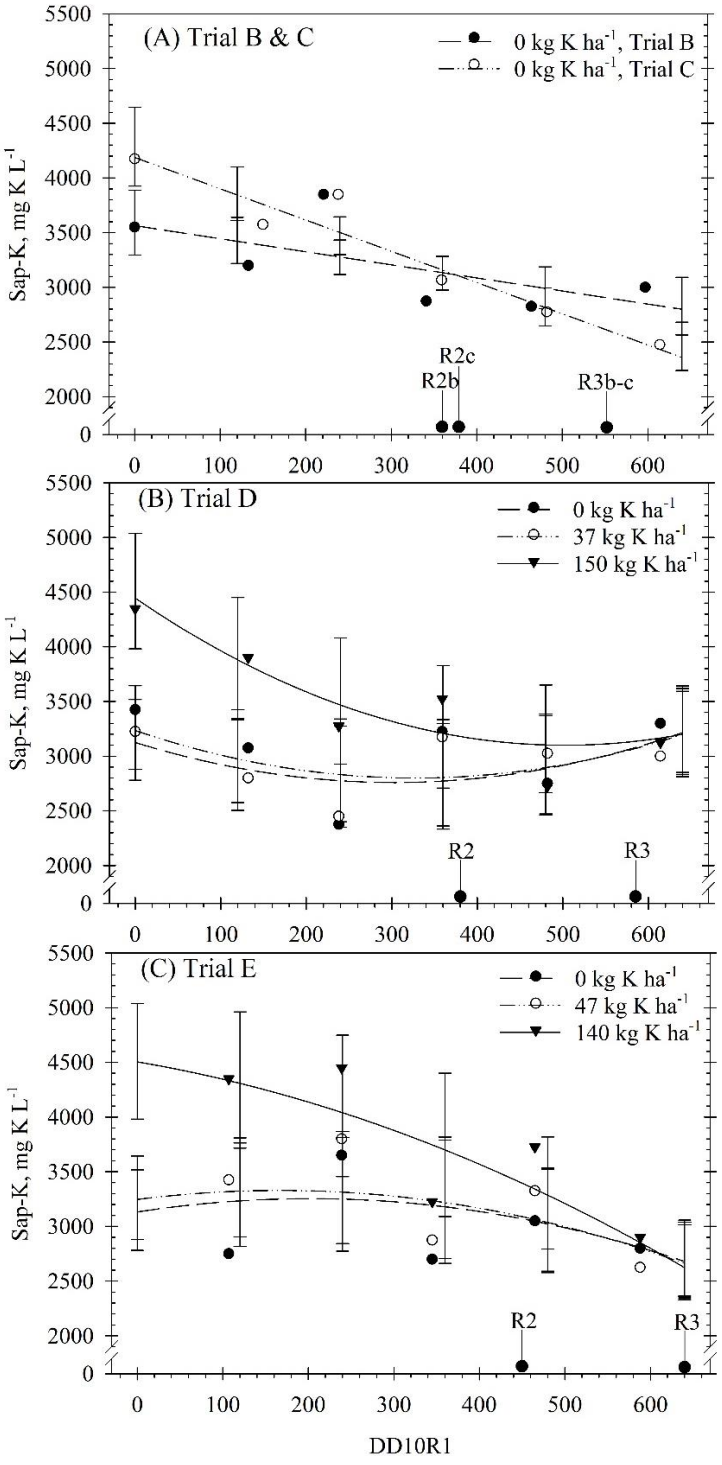

Fig. 3.3. Sap-K analyzed with HKIM for short-term pure-line trials B and D and hybrid trials C and E with error bars at 0, 120, 240, 360, 480 and 640 DD10R1 that allowed for comparisons between cultivars, across points in time, and among fertilizer K rates. Adjacent trials were B and C (Fig. 3.3A) and D (Fig. 3.3B) and E (Fig. 3.3C). All trials were located at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR during 2018. Regression coefficients are shown in Table 3.6.

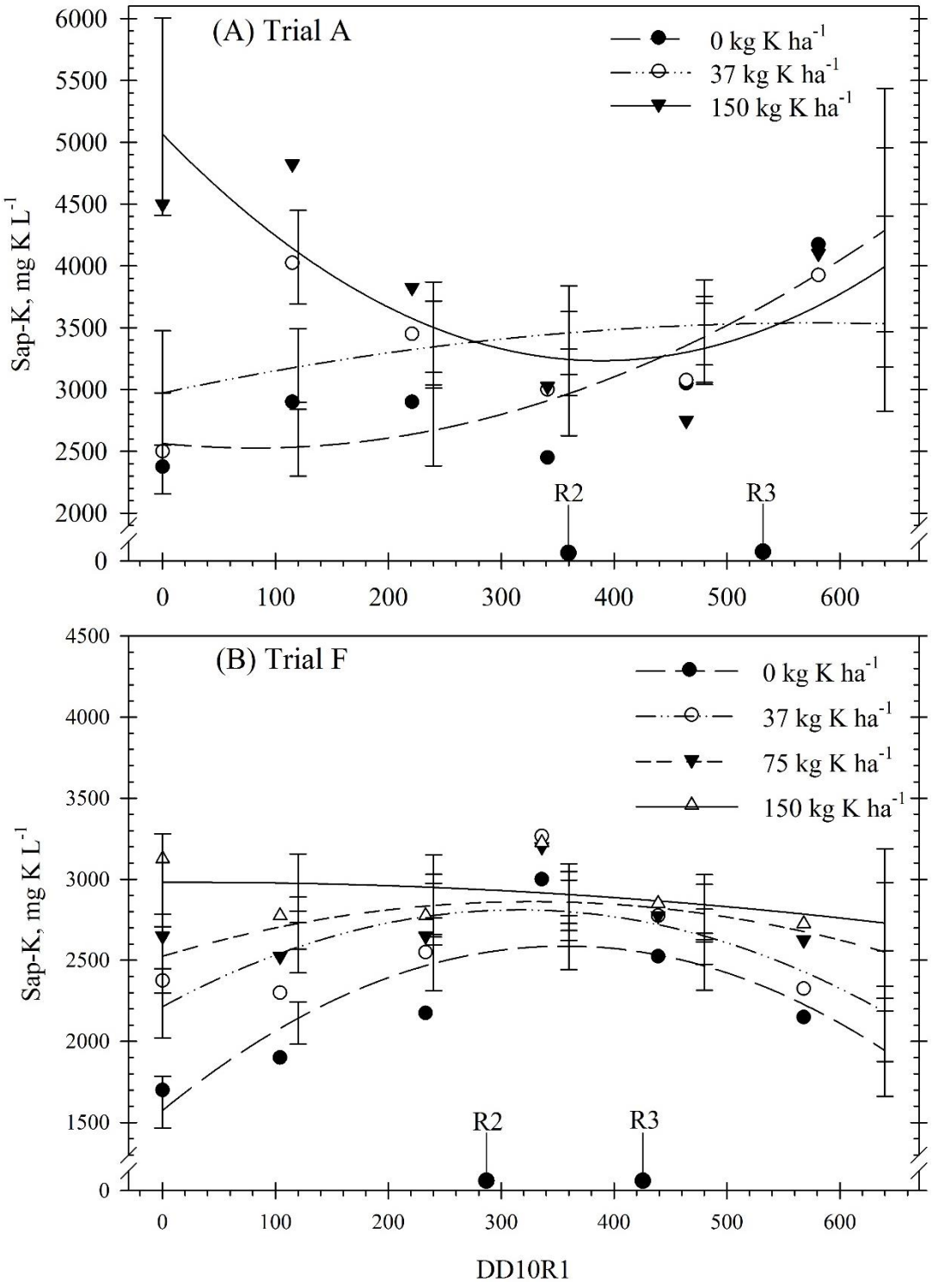

Fig. 3.4. Comparisons of sap-K for long-term pure-line (Trial A & F) trials among fertilizer K-rates along with within K-rates across time using regression with error bars at 0, 120, 240, 360, 480 and 640 DD10R1 allow comparison among K rates and across points in time. Trials are located at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR during 2018 or 2019. Regression coefficients are shown in Table 3.6.

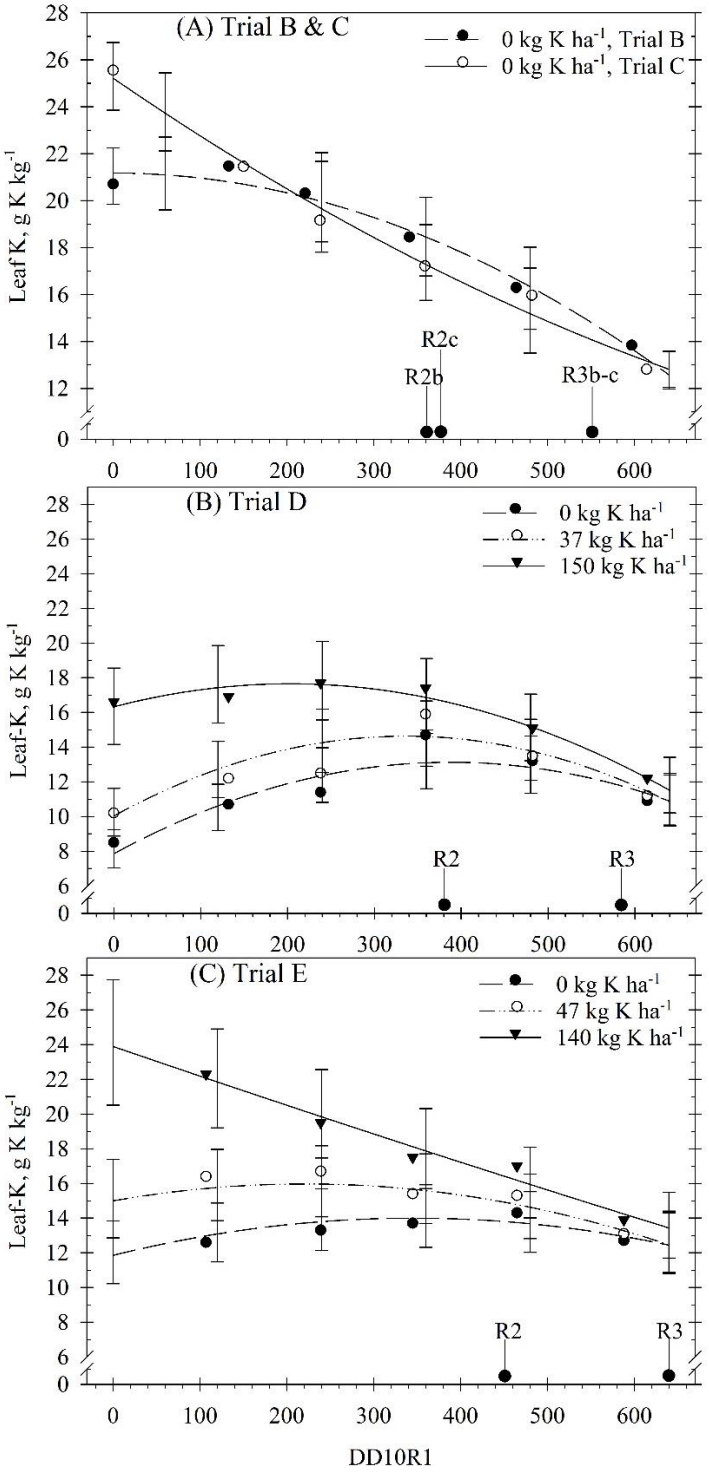

Fig. 3.5. Leaf-K for short-term pure-line trials B and D and hybrid trials C and E with error bars at 0, 120, 240, 360, 480 and 640 DD10R1 that allowed for comparisons between cultivars, across points in time, and among fertilizer K rates. Adjacent trials were B and C (Fig. 3.3A) and D (Fig. 3.3B) and E (Fig. 3.3C). All trials were located at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR during 2018. Regression coefficients are shown in Table 3.7.

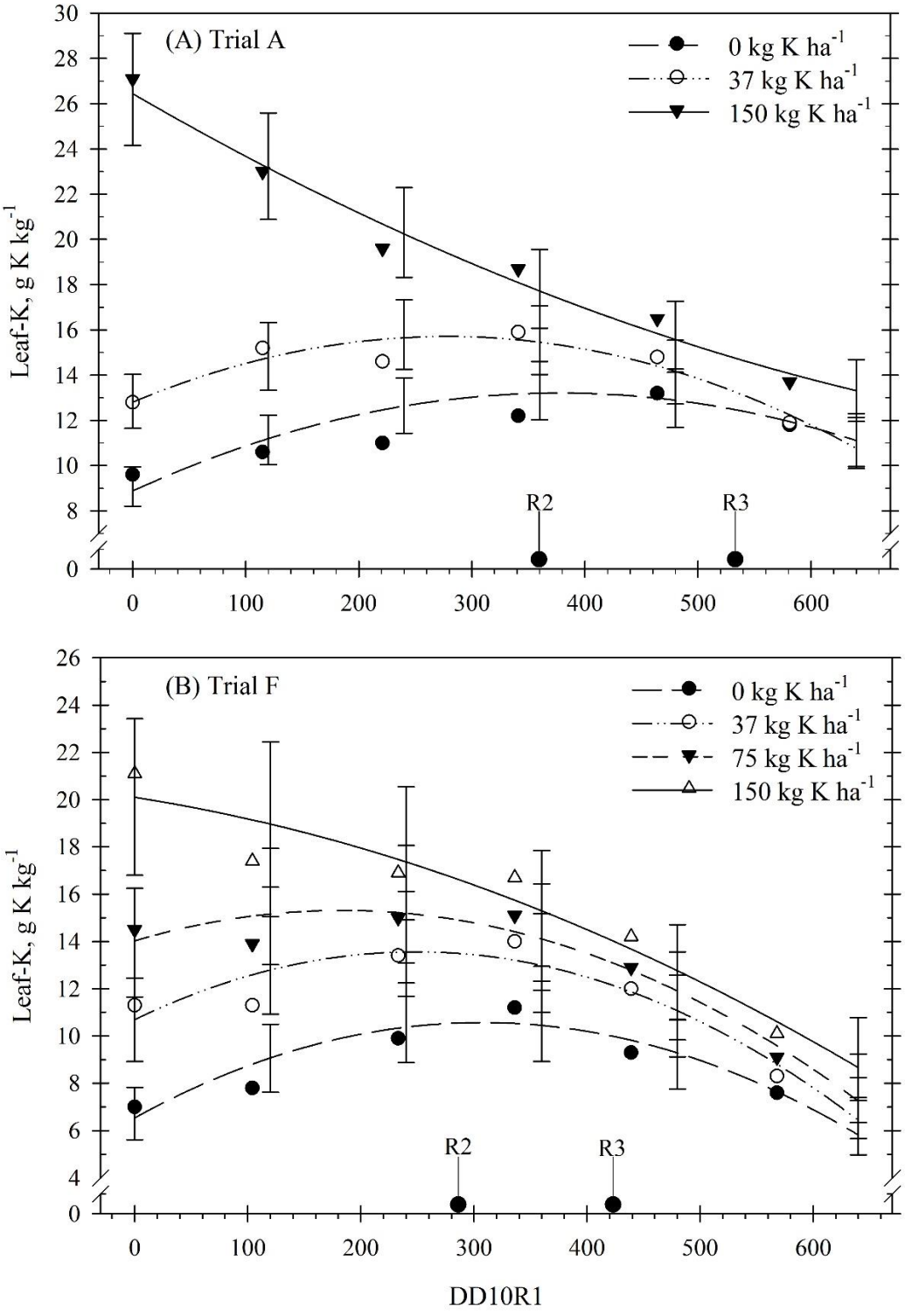

Fig. 3.6. Leaf-K comparisons for long-term pure-line (Trial A & F) trials among fertilizer K-rates and also within K-rates across time using regression with error bars at 0, 120, 240, 360, 480, and 640 DD10R1 allow comparison among K rates and across points in time. Trials are located at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR during 2018 or 2019. Regression coefficients are shown in Table 3.7.

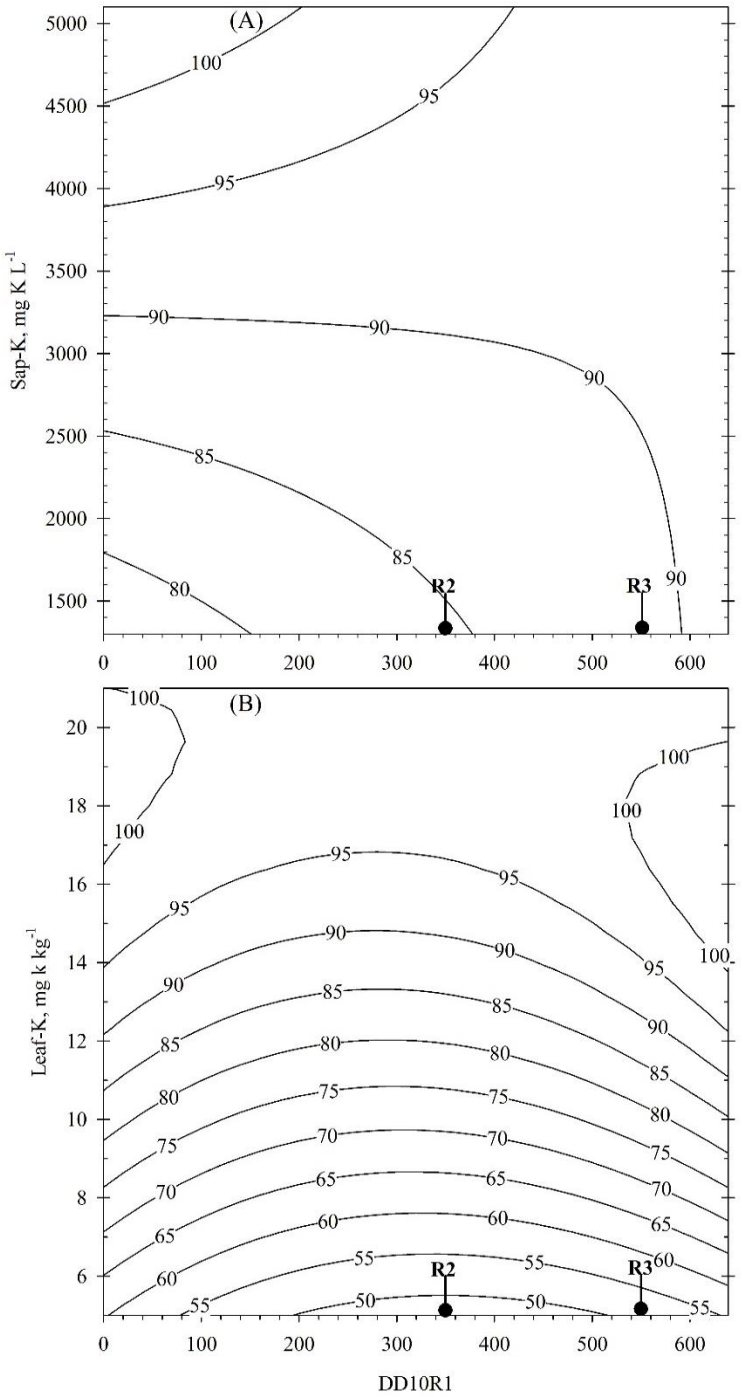

Fig 3.7. Relative yield predictions as affected by the cumulative number of growing degree units after the R1 stage (DD10R1) and sap-K (A) or Y-leaf-K (Leaf-K; B) concentration as measured on a handheld Horiba K^+ ion meter (HKIM; A) or traditional leaf analysis (B) using only pure-line cultivar data from Trials A, B, D, and F located at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR during 2018 or 2019.

Conclusion

Our research suggested that the grain yield of hybrid cultivar, Gemini 214 Clearfield, did not respond to K fertilization, but the grain yield of pure-line cultivars, Diamond and CL 153, responded positively to K fertilization in five of eight trials on soils having suboptimal soil-test K. We developed a continuous critical Y-leaf-K concentration curve that can be used to assess te K nutritional status of pure-line rice cultivars from the R1 through the R3 growth stages. The hybrid rice had Y-leaf-K concentrations that were generally similar across time to the Y-leaf-K concentration of the hybrid cultivar. These data suggest that the hybrid cultivar Gemini 214 Clearfield and perhaps other hybrid cultivars may be less sensitive to K deficiency and responsive to K fertilization compared to pure-line cultivars. The sap-K concentration of the Y-leaf as determined on the Horiba K ion meter was weakly related ($R^2 = 0.39$) to the Y-leaf-K concentration following digestion in nitric acid and determined by inductively coupled plasma atomic emission spectroscopy. The sap-K concentration showed no consistent trend across time among sites or fertilizer-K rates and was poorly correlated to rice grain yield, regardless of cultivar type.

Appendix

Appendix 2.1. Maximum (Max) and minimum (Min.) Y-leaf-K concentrations and the cumulative growing degree units after the R1 growth stage by K-rate and trial calculated using the back-transformed predicted values from quadratic equation coefficients in Table 2.4 for 13 trials located at the University of Arkansas System Division of Agriculture Pine Tree Research Station (PTRS) near Colt, AR and the Rice Research and Extension Center (RREC) near Stuttgart, AR in 2018 and 2019.

Site-year	Fertilizer-K rate	Intercept[a]	Linear	Quadratic	Max Leaf-K	Max Leaf-K time	Min. Leaf-K	Min. Leaf-K time
	kg K ha^{-1}							
PTRS-18a	0	8.88	0.0230	-0.000031	13.22	377	9.03	0
	37	12.81	0.0209	-0.000038	15.71	277	10.94	640
	150	26.45	-0.0290	0.000013	26.52	0	13.25	640
PTRS-18b[b]	0	21.18	0.0000	-0.000021	21.18	0	11.99	640
PTRS-18c	0	25.22	-0.0255	0.000010	25.22	0	12.05	640
PTRS-18d	0	7.85	0.0270	-0.000035	13.03	384	8.08	0
	37	10.03	0.0280	-0.000041	14.82	342	10.16	0
	150	16.32	0.0130	-0.000032	17.33	201	11.70	640
PTRS-18e	0	11.85	0.0120	-0.000018	13.85[c]	334	11.89[c]	0
	47	14.99	0.0088	-0.000020	15.97[c]	221	13.45[c]	640
	140	23.89	-0.0170	0.000001	23.86	0	13.46	640
PTRS-19a	0	6.54	0.0263	-0.000043	10.58	307	6.02	640
	37	10.66	0.0230	-0.000047	13.50	247	6.84	640
	75	14.02	0.0140	-0.000039	15.29	181	7.65	640
	150	20.11	-0.0076	-0.000016	19.84	0	8.93	640
PTRS-19b	0	12.58	0.0087	-0.000020	13.52[c]	217	10.05[c]	640
	37	14.98	0.0039	-0.000017	15.19	109	11.84	640
	150	21.21	-0.0143	-0.000004	21.11[c]	0	10.57[c]	640
PTRS-19c	0	13.30	-0.0005	-0.000005	13.31	50	11.04	640
	47	15.79	-0.0063	-0.000001	15.78	0	11.35	640
	140	22.25	-0.0264	0.000015	22.36	0	11.61	640
PTRS-19d	0	12.12	0.0160	-0.000032	14.10	247	9.28	640
	37	15.01	0.0073	-0.000027	15.52	138	9.04	640
	150	19.29	-0.0103	-0.000008	19.16	0	9.72	640

Appendix 2.1 (Cont.)

Site-year	Fertilizer-K rate	Intercept[a]	Linear	Quadratic	Max Leaf-K	Max Leaf-K time	Min. Leaf-K	Min. Leaf-K time
	kg K ha^{-1}							
PTRS-19e	0	13.48	0.0074	-0.000022	14.11	171	9.49	640
	47	16.63	-0.0027	-0.000014	16.49	0	9.24	640
	140	21.29	-0.0223	0.000007	21.29	0	9.94	640
PTRS-19f	0	14.95	0.0177	-0.000040	16.92	220	10.10	640
	150	22.63	-0.0058	-0.000021	22.35	0	10.77	640
PTRS-19g	0	18.04	-0.0005	-0.000018	17.86	0	10.50	640
	140	23.44	-0.0222	-0.000005	23.40	0	11.15	640
RREC-19	0	16.63	0.0041	-0.000019	16.86	112	11.86	640
	37	22.35	-0.0120	-0.000005	22.26	50	12.77	640
	150	31.42	-0.0409	0.000020	31.54	0	13.26	640

[a]Coefficients have been derived from the regression in Fig. 2.1-2.3.
[b]Adjacent trials(PTRS-18b and PTRS-18c, PTRS-18d and PTRS-18e, PTRS-19b and PTRS-19c, PTRS-19d and PTRS-19e, PTRS-19f and PTRS-19g).
[c]Leaf-K maximum (Max) and minimum (Min.) values that are not significantly different from each other at the 0.05.